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(57) Abstract

This invention relates to the therapeutic induction of apoptosis in activated inflammatory cells, or cells at a site of inflammation, by introducing into those cells a chimeric gene containing an apoptosis–inducing gene (AIG) driven by a promoter of an inducible gene activated in inflammation and a promoter enhancer such that the inflammatory cells are targeted. In one embodiment, the chimeric gene comprises at least one TNFα promoter enhancer attached to a functional copy of a minimal TNFα promoter and further attached to at least one copy of an apoptosis–inducing gene, wherein expression of the gene is driven by the TNFα promoter. Attachment can be direct, distal, proximal or combinations thereof. Example apoptosis–inducing genes include caspase 3, caspase 4, caspase 5, Granzyme B. Advantageously, the TNFp–AIG chimeric gene is expressed in only those cells producing the inflammatory cytokine, TNFα. In addition, the TNFp–AIG chimeric gene also sequesters inducible TNFp transcription factors, thereby reducing endogenous production of TNFα. The invention also relates to methods of making and using self–regulated apoptosis chimeric genes and pharmaceutical compositions containing them for treating inflammatory diseases.

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SELF-REGULATED APOPTOSIS OF INFLAMMATORY CELLS BY GENE THERAPY

Related Application Data

This application claims priority benefit of co-pending U.S. provisional pplication serial number 60/039,266, filed February 28, 1997.

Technical Field of the Invention

This invention relates to the therapeutic induction of apoptosis in inflammatory cells by introducing into those cells a gene which induces apoptosis (programmed cell death or non-necrotic cell death) in these cells. The Apoptosis-Inducing Gene (which will sometimes be referred to herein as AIG) is driven by a TNFα promoter (TNFp) or other inducible gene activated in inflammation. In one embodiment, apoptosis is selectively induced in those cells capable of producing TNFα. The TNFp-AIG or other chimeric gene may be conveniently introduced *in vivo* using conventional gene therapy techniques. Advantageously, in the embodiment wherein the chimeric gene is TNFp-AIG, it is expressed in only those cells producing the inflammatory cytokine, TNFα. In addition, since the TNFp-AIG chimeric gene contains the TNFα promoter elements, it also sequesters inducible, TNFp-selective transcription factors. Such sequestration results in a reduction in endogenous production of TNFα. The present invention relates specifically to TNFp-AIG and similar gene constructs, cells containing chimeric genes, methods for induction of apoptosis in cells transfected with chimeric genes, pharmaceutical compositions containing chimeric genes, methods

for *in vitro* selection of TNFα non-producer somatic cell variants within a TNFα producing cell population and the like, a method for identifying dominant negative/dominant suppressive genes responsible for inhibiting TNFα production and therapeutic methods using the chimeric gene.

Background of the Invention

In many inflammatory conditions, cytokines such as IL-1, IL-10, GM-CSF and TNFα are excessively produced as a result of mass aggregation and accumulation of inflammatory cells (Brennan F.M. *et al., British Medical Bulletin* 1995, 51/2, 368-384). Upregulation and/or disregulation of cytokines in inflamed tissue may be directly or indirectly responsible for exacerbation of chronic inflammatory diseases. For example, the most marked pathology in rheumatoid arthritis (RA) is displayed at the local site of inflammation (*i.e.*, the synovial joints). Therefore, it is likely that the cytokines produced in the synovial joints of RA patients play an important role in the disease process. Of those cytokines, IL-1 and TNFα are believed to be responsible for the devastating cartilage destruction and bone erosion which characterizes RA (Dayer J.M. *et al., J. Exp. Med.*, 1985, 162, 1208-1215; Gowen M. *et al., Nature*, 1983, 306, 378-380). The presence of excessive amounts of IL-1 and TNFα in the synovial joints has been shown to accelerate development of collagen-induced arthritis in rodents (Brennan F.M., *et al., Clin. Expt. Immunol.*, 1994, 97/1, 1-3). Excessive amounts of TNFα and IL-1 are produced in the synovial tissue by a variety of cell types at the cartilage-pannus junction, including cells of the macrophage lineage, macrophage-like synoviocytes, activated T-cells and possibly fibroblast-like synoviocytes (Chu C.Q. *et al., Arthritis & Rheumatism*, 1991, 34, 1125-1132; Deleuran B.W., *et al., Arthritis & Rheumatism*, 1992, 35, 1170-1178).

In addition to the above described inflammatory effects, TNFα plays a ubiquitous and key role in a variety of pro-inflammatory events, such as induction

of IL-1 activity in monocytes. Indeed, anti-TNFα neutralizing antibodies have been shown to reduce overall IL-1 production (Portillo, *et al., Immunol.*, 1989, 66, 170-175; Brennan F.M., *et al., British Medical Bulletin* 1995, 51/2, 368-384). Thus, an added benefit to blocking the effect of the inflammatory cytokine TNFα is the reduction in production of the equally destructive pro-inflammatory mediator, IL-1. Furthermore, it is well known that TNFα is a transcriptional activator of other inflammation-related genes. For example, the presence of TNFα stimulates production of other cytokines (such as GM-CSF) and cell surface receptors, including HLA class II antigens and adhesion molecules (Alvaro-Garcia J.M., *et al., J. Exp. Med.*, 1989, 146, 865-875), which. results in continuous recruitment of activated T cells and neutrophils resulting in synovial inflammation and hyperplasia and ultimately, in augmented destruction of cartilage and bone (Allen J.B., *J. Exp. Med.*, 1990, 171, 231).

Conventional therapy against inflammatory disorders is typically directed against symptomatic inflammation. Such therapies provide only temporary relief without significantly delaying disease progression. In contrast, therapies targeting TNFα and other factors induced in the inflammatory process are likely to be more promising. For example, in collagen-induced arthritis animal models, an anti-TNFα antibody and soluble TNFα receptor-IgG chimera effectively reduced paw swelling, joint involvement and cartilage and bone destruction (Williams R.O. *et al., Proc. Natl. Acad. Sci.*, 1992, 89, 9784-9788). Human trials using both humanized anti-TNFα antibodies and TNFα receptor-IgG chimeric molecules produced dramatic results (Elliott M.J., *et al., Arthritis and Rheumatism*, 1993, 36, 1681-1690; Elliott M.J., *et al., Lancet*, 343, 1105-1110). Although treatment with these TNFα antagonists appears to be well tolerated, it also results in production of antibodies against the recombinant proteins. Thus, these therapies may not be suitable for long term treatment and do not achieve true disease abatement. In order to actually modify progression of the disease, TNFα must be continuously targeted using TNFα-specific therapies. Such a therapeutic protocol

is impractical with these biologic agents and would be difficult to administer in the long term.

In an alternate therapeutic option, inflamed synovium may be removed using surgical (Herold N. and Schroder H.A., *Acta Orthop. Scand.*, 1995, 66, 252-254; Ogilvie-Harris D.J. and Weisleder L., *Arthroscopy*, 1995, 11, 91-95), chemical (Cruz-Esteban C. and Wilke W.S., *Bailliere's Clinical Rheumatol.*, 1995, 9, 787-801) or radiation-induced synovectomy (Cruz-Esteban C. and Wilke W.S., *Bailliere's Clinical Rheumatol.*, 1995, 9, 787-801). The results following arthroscopic surgical synovectomy are good, showing improvement from the preoperative condition to the postoperative condition. Non-surgical synovectomy is performed using various chemical agents such as osmic acid, alkylating agents such as nitrogen mustard and thiotepa, methotrexate. Unfortunately, non-surgical synovectomies (including chemical and radiation-induced) are procedurally complicated, provide only short term relief and show only patchy reduction of the synovial hyperplasia. Furthermore, most of the non-surgical alternatives are potential teratogens. In addition, the chemical damage to afflicted tissue in non-surgical synovectomy, as well as surgically-induced tissue damage, often cause an inflammatory response themselves. Finally, it should be noted that these approaches suffer from the risks and side-effects commonly associated with conventional pharmaceutical therapy and invasive surgical procedures, including the expense and inconvenience of hospitalization and rehabilitation.

Accordingly, a need still exists for an effective therapeutic approach to treating inflammatory disorders in general and RA in particular.

Summary of the Invention

This invention overcomes the drawbacks associated with previous therapies for treating inflammatory disorders by providing a novel therapeutic

approach. According to one embodiment of this invention, apoptosis is selectively induced in TNFα-producing inflammatory cells, causing destruction of these cells without an associated inflammatory reaction.

One objective of this invention is to provide a therapeutic method comprising the step of introducing into the inflammatory cells of a mammal, or cells at a site of inflammation, a chimeric gene containing a self-regulating apoptosis-inducing gene (AIG). The AIG is driven by a promoter such as a TNFα promoter (TNFp; see Figures 1 and 2), and, preferably, a promoter enhancer. Therefore, it is expressed in all and only those cells capable of producing TNFα.

Another objective of this invention is to provide TNFp-AIG and the like chimeric gene constructs, processes for making them, methods of using them, and preparations containing them.

Yet a further objective of this invention is to provide a method for the induction of apoptosis in cells transfected with the TNFp-AIG chimeric gene, a method for the *in vitro* selection of TNFα non-producer somatic cell variants in a population, a method for identifying dominant/negative genes responsible for the genesis of a TNFα non-producing population and a method for identifying products responsible for regulation of TNFα production (Figure 10).

These and other objectives will be readily appreciated by those of ordinary skill in the art based upon the following detailed disclosure of the invention.

Brief Description of the Figures

Figure 1 is a schematic representation of TNFp-AIG chimeric genes of this invention. Apoptosis Inducing Gene (AIG) could be any, but not limited to, the genes listed, *viz.*, Caspases 1 to 10, Granzyme B, FasLigand, *etc.*

Figure 2 is a schematic drawing depicting the results of gene therapy using a TNFp-AIG chimeric genes of this invention.

Figure 3 is a summary of deletion constructs used for identification of the inducible cis elements of the TNFα promoter using luciferase gene (Luc) expression as the reporter system.

Figure 4 (a and b) provide a summary of results obtained using the constructs described in Figure 3. Transient expression of the constructs was assessed in two different TNFα-producing cell lines, *viz.*, Jurkat (Figure 4a) and THP-1 (Figure 4b). Histograms in each figure show stimulation index as a measure of inducibility by activating agents such as PMA (Figure 4a) or LPS (Figure 4b) for individual experiments. The line superimposed in each figure indicates the mean inducibility averaged from 4 to 6 experiments.

Figure 5 is a flow chart for preparation of the TNFpAIG using selected native elements of the TNFα promoter and prodomain-deleted AIGs (AIGs used are Caspase and Caspase 4/5).

Figure 6 (a, b, and c) provide a summary of results from representative experiments performed to see expression of the chimeric TNFpAIGs. Apoptosis in transiently-transfected Jurkat cells (Figure 6a and 6b) and THP-1 (Figure 6c) cells was assessed using Cell Death Elisa (CDE assay). In all three figures, histograms with sparse dots represent transfection control, where cells were treated with the transfecting agent in the absence of DNA. Histograms with dense dots represent the TNFp elements driving expression of the luciferase gene and solid histograms represent the same TNFp elements driving expression of either AIG.1 or AIG.2. The number in parenthesis above the solid histograms represent enrichment factor (ratio of apoptosis induced by TNFpAIG to the TNFpLuc control vector).

Figure 7 (a and b) is a diagrammatic representation of a TNFp-AIG chimeric gene of this invention, comprising multiple copies of the inducible cis elements of the TNFα promoter which, in turn, drive expression of the AIG (Figure 7a). A diagrammatic representation of a TNFpAIG chimeric gene, comprising multiple copies of the inducible cis elements of the TNFα promoter, driving expression of the AIG, downstream of which are 3' untranslated region of the TNFα gene (TNF3'UTR) (Figure 7b). 3'UTR of the TNFα gene is implicated in the regulation of the inducible expression of TNFα (Han, J., *et al., J. Immunology*, 1991, 146, 1843-1843, Crawford, E.K., *et al., J. Biol. Chem.*, 1997, 272, 21120-21137, and Figure 9).

Figure 8 (a and b) are flow charts of schemes for preparing TNFα superpromoter-AIG chimeric constructs.

Figure 9 shows a summary of the results of two experiments to show the regulatory effect of the TNF3'UTR on inducible expression of the luciferase reporter gene. The transient transfection was performed in a fibroblast cell line. Dotted histograms represent inducibility of TNFpLuc in the absence of TNF3'UTR and solid histograms represent inducibility of TNFpLuc in the presence of TNF3'UTR. Similar results are obtained in the Jurkat cell.

Figure 10 is a diagrammatic representation for the selection of TNFα non-producer somatic cell variants within a TNFα-producing cell population and identification of dominant negative suppressive genes responsible for inhibiting TNF-α production.

Detailed Description of the Invention

This invention is based upon evidence that apotosis of inflammatory cells in certain inflammatory diseases is therapeutically beneficial. The invention

specifically relates to self-regulated apoptosis by gene therapy. Broadly speaking, in the practice of the invention, a chimeric gene comprising at least one promoter enhancer attached to at least one functional copy of a minimal promoter, the promoter being a gene or combination of genes activated in inflammatory cells or in cells at a site of inflammation, is attached to at least one copy of an apoptosis-inducing gene (AIG), such that the expression of the apoptosis-inducing gene is driven by the promoter, thus targeting the inflammatory cells. Example promoters of inducible genes activated in inflammation include, but are not limited to, cytokines, interleukins and their receptors, cell adhesion molecules and their ligands, chemokines and their receptors, pro-inflammatory enzymes, and the like. Chimeric genes according to the invention comprise enhancer, promoter, and AIG elements in direct, distal, or proximal attachment, and combinations thereof. As mentioned above and will be discussed in more detail below, in some embodiments, multiple copies of the enhancer, promoter, and/or AIG are employed for maximal efficacy.

In order that the invention herein described may be more fully understood, the following detailed description is set forth, with emphasis on chimeric genes comprising at least one TNFα promoter enhancer attached to at least one functional copy of a minimal TNFα promoter and further attached to at least one copy of an AIG for illustrative purposes only. Though the examples that follow also employ these types of constructions, it will be appreciated by skilled workers that the basic constructs described herein may be altered to provide other embodiments that utilize products, processes, methods, and compositions of the invention with other promoters comprising inducible genes activated in inflammation such as the types listed above that exhibit similar functions that can be used to target cells at the site of infection.

For example, cytokines and interleukins useful as promoters in the construction of chimeric genes of the invention include, but are not limited to, TNF-α, TNFβ, IL-1α, IL-1β, IL-2, Il-6, IL-9, GM-CSF, interferonγ, and the

like, and functional fragments and mixtures thereof. Cell adhesion molecules and their ligands include, but are not limited to, selectins, integrins, and members of the immunoglobulin superfamily such as ICAM-1, V-CAM, and the like, and functional fragments and variants and mixtures thereof. Chemokines and their receptors include, but are not limited to, the C-X-C and C-C family members such as MIP-1α, MIP-1β, MCP1-4, RANTES, Mig, NAP2, IP10, Gro α-γ and the like, and functional fragments and variants and mixtures thereof. Pro-inflammatory enzymes include, but are not limited to COX-2, iNOS, phospholipases, proteases (including matrix metalloproteases), and the like and functional fragments and mixtures thereof.

To clarify the discussion below of exemplary TNFp-AIG chimeric genes of this invention, the following sequences are illustrated:

SEQ ID NO: 1 is the nucleotide sequence corresponding to the full-length, reference human TNFα promoter sequence, as published in (Takashiba S., *et al., Gene*, 1993, 131, 307-308). Nucleotide numbers used herein refer to the numbering of this sequence.

SEQ ID NO: 2 is the native TNFα promoter sequence of the gene that was used in this invention (-1077 nucleotides from the transcription start site, TSS). There are a few differences in the sequence of the TNFp in SEQ ID NO: 1 and SEQ ID NO: 2. Such differences in the nucleotide sequences of the TNFα promoter have been reported (Takashiba S., *et al., Gene*, 1993, 131, 307-308).

SEQ ID NO: 3 is the native minimal TNFα promoter sequence (nucleotide -120 through -TSS, which includes at least one enhancer element (k1 site; see Pauli, U., *Crit. Rev. in Eucaryotic Gene Expression*, 1994, 4, 323-344; Rhoades K.L., *et al., J. Biol. Chem.*, 1992, 267, 22102-22107; and Takashiba S., *et al., Gene*, 131, 307-108).

SEQ ID NO: 4 is the chimeric gene TNFp120 AIG.1 (containing -120 TNFp driving the expression of the prodomain-deleted variant of CPP32 gene (Caspase 3, published Tewari M. *et al., Cell*, 1995, 81(5), 801-809, with the variation being V239A).

SEQ ID NO: 5 is the chimeric gene TNFp706 AIG.1 (containing -706TNFp driving expression of the prodomain-deleted CPP 32 gene.

SEQ ID NO: 6 is the TNFp1005 AIG.1 (containing -1005 TNFp driving expression of the prodomain-deleted CPP 32 gene).

SEQ ID NO: 7 is the chimeric gene TNFp120 AIG.2 (containing -120 TNFp driving expression of the prodomain-deleted Ty/x gene. (Sequences of Ty (Caspase 5) and Tx (Caspase 4) genes are published in the ref. Faucheu, C., *et. al., Eur. J Biochem.*, 236, 207-213, 1996; Faucheu, C., *et. al. EMBO J.*, 14, 1914-1922,1995).

SEQ ID NO: 8 is the chimeric gene TNFp706 AIG.2 (containing -706TNFp driving expression of the prodomain-deleted Ty/x gene.

SEQ ID NO: 9 is the TNFp1005 AIG.1 (containing -1005 TNFp driving expression of the prodomain-deleted Ty/x gene).

SEQ ID NO: 10 is the enhancer region 1 (ER1) of the TNFα promoter encompassing nucleotides -1005 to -905.

SEQ ID NO: 11 is the enhancer region 2 (ER2) of the TNFα promoter encompassing nucleotides -706 to -517.

SEQ ID NO: 12 is additional multiple cloning sites (MCS) genetically engineered upstream of the -120 minimal TNFα promoter in the -120pGL3 construct.

SEQ ID NO: 13 is the 3' untranslated region (3'UTR) of the TNFα gene (Nedwin, G.E., *et al., Nucleic Acid Research*, 1985, 13, 6361-6373).

The elements of the TNFα promoter for preparation of chimeric gene constructs according to this invention are selected from elements which are capable of inducing expression of a therapeutic gene driven by the TNFα promoter. These promoter elements will be referred to herein as "inducible cis elements", "cis-inducible elements" or "enhancer elements" of the TNFα promoter.

The enhancer elements may be physically linked to the minimal promoter sequence, or separated from the minimal promoter by a linker sequence which may or may not have unique restriction sites. Thus, as summarized above, enhancer elements may be attached directly, distally, proximally, or any combination thereof, to chimeric genes of the invention. These are typically constructed upstream of the promoter. Example TNFα enhancer elements are set out in SEQ ID NO: 10 and SEQ ID NO: 11; functional fragments or variants and combinations thereof may be employed. Some preferred gene constructs according to this invention include those that have multiple copies of the enhancer elements, *i.e.*, 2 or more copies. Some embodiments have about 2 to 25, more narrowly 2 to 10, and even more narrowly, 2 to 5 copies.

The terms "TNF promoter", "TNFα promoter" and "TNFp" are used interchangeably herein. Unless noted to the contrary, these terms refer to the entire nucleotide sequence corresponding to a native TNFα minimal promoter sequence attached to one or more upstream enhancer elements (either present naturally i.e. native, or genetically engineered in the laboratory). Examples include, but are not limited to, SEQ ID NO: 1, SEQ ID NO: 2, and SEQ ID NO: 3, and functional fragments, variants, and mixtures of any of these. Many

functional fragments and variants of these TNFα sequences and others described herein share a sequence homology of at least about 80%, and in some cases over 90%, to their native and genetically engineered counterparts, but these are known to skilled workers and defined in the references cited herein.

Any apoptosis-inducing gene may be used in the chimeric genes and methods described herein. The apoptosis inducing gene used for the chimeric therapeutic genes of this invention can be the same or different from the type of apoptosis inducing gene present in the native sequence of the TNF-α-producing inflammatory cells (if those cells naturally contain an apoptotic gene). Preferred AIGs include, but are not limited to, members of the ICE/CED3 family of apoptosis inducing proteases (such as Caspase-1 (ICE), hICE, ICE-LAP45, Mch2α), Caspase-2 (ICH1), Caspase-3 (CPP32, Yama, Apopain), Caspase-4 (TX, ICH2, ICE rel II), Caspase-5 (ICE rel III, TY), Caspase-6 (Mch-2), Caspase-7 (Mch-3, ICE-LAP3, CMH-1), Caspase-8 (MACH, FLICE, Mch-5), Caspase-9 (ICE-LAP6, Mch6) and Caspase-10 (Mch4)), members of the granzyme family (such as Granzyme A and Granzyme B), Fas ligand (FasL), and functional fragments, variants, and mixtures of any of these. Some embodiments employ Caspase 3, Caspase 4, Caspase 5, Granzyme B, and functional fragments, variants, and mixtures thereof. With the exception of FasL, these genes, when overexpressed following transfection, induce apoptosis in the transfected cells (Miura M., *et al., Cell*, 1993, 75, 653-660; Chinnayan A.M., *et al., Cell*, 1995, 81, 505-512; Los, *et al., Nature*, 1995, 375, 81; Muzio, *et al., Cell*, 1996, 85, 817-827).

In the case of FasL, apoptosis is induced (either in an autocrine or a paracrine fashion) in only those cells that express Fas. Therefore, the TNFp-FasL chimeric gene construct offers a second level of selectivity. Another advantage of the TNFp-FasL chimeric gene is the selective targeting of those disease-producing cells in the synovium that do not express TNFα (thereby failing to drive expression of the apoptosis inducing gene), but do express Fas on the surface. In this

case, FasL will be expressed by the cells that are capable of producing TNFα such as activated macrophages and T cells. These cells will then induce apoptosis in Fas-expressing cells such as hazardous activated T cells and Fas-expressing synoviocytes.

This invention provides a novel therapeutic method comprising the step of introducing into the cells of a mammal a chimeric gene comprising an apoptosis-inducing gene (AIG) driven by the TNFα promoter (TNFp). Example chimeric genes of the invention are set out in SEQ ID NOs 4, 5, 6, 7, 8, and 9; functional fragments or variants of these may also be employed. Without wishing to be bound by theory, as a result of being controlled by the TNFp, AIG is expressed in only those cells producing the inflammatory cytokine, TNFα. Therefore, any cells expressing TNFα will be self-destructive, while cells that do not express TNF-α will be unaffected. Advantageously, this methodology can target any TNFα-producing cells (such as activated macrophages, activated T-cells and macrophage-like and possibly fibroblast-like synoviocytes) without regard to cell type. Indeed, the targeted TNFα-producing cell can be one which normally does or normally does not carry or expresses an apoptosis gene in its native, unaltered form. Therefore, using the chimeric genes and methods of this invention, the cellular sources of TNFα can be destroyed in a highly selective manner.

Another advantage of using the TNFp-AIG chimeric gene of this invention is that TNFp sequesters transcription factors needed by endogenous TNFp, thereby leading to a reduction in endogenous TNFα production. In one preferred embodiment, TNFp is present in the therapeutically targeted cell in vast excess. This may be accomplished by introducing multiple copies of the transfected gene into the cell. Alternatively, the TNFp-AIG chimeric gene according to this invention can contain multiple copies of the inducible cis elements of the TNFα promoter. As mentioned above, multiple copies of the "inducible enhancer elements" of TNFp are present in some embodiments of the TNFp-AIG chimeric genes of this invention. By including multiple copies of the inducible cis elements

of the TNFp construct, the transcriptional factors needed by the transfected cell to produce TNFα are sequestered by the exogenously introduced sequence. This preferred chimeric TNFp-AIG construct is characterized by an increased effectiveness in competing for the TNFp-specific transcription factors as compared to chimeric genes of this invention containing only a single enhancer element linked to TNFp. The "inducible super promoter" constructed in this way is capable of (1) more effectively competing for TNFα specific inducible transcription factors and (2) driving expression of the apoptosis inducing gene in an augmented fashion by virtue of multiple enhancing elements.

For example, in rheumatoid arthritis patients, synovectomy, *i.e.*, removal of synovial tissue, has been shown to be clinically beneficial. Unlike conventional and surgical synovectomy procedures, the cell-targeted therapeutic method described herein targets only cells producing TNFα. Thus, advantageously, the introduction and expression of the TNFp-AIG chimeric gene, and subsequent induction of apoptosis do not induce an inflammatory response. Accordingly, methods of this invention are comparatively selective and result in minimal tissue damage and a reduction in inflammation.

The products and methods described herein are useful for the treatment of other inflammatory disorders as well. Such inflammatory disorders include, but are not limited to, multiple sclerosis, Guillain-Barre syndrome, Crohn's disease, ulcerative colitis, psoriasis, graft versus host disease, lupus erythematosus, insulin-dependent diabetes mellitus, psoriatic arthritis, sarcoidosis, hypersensivity pneumonitis, ankylosing spondylitis and related spoldyloarthropathies, Reiter's syndrome and systemic sclerosis. Thus, this invention encompasses methods for treating an inflammatory disorder in a patient by inducing apoptosis in inflammatory cells or cells at a site of inflammation of the patient by introducing into the cells at least one chimeric gene of the invention. This is typically accomplished by preparing a pharmaceutical composition containing at least one chimeric gene of the invention and typically a pharmaceutically acceptal carrier, and administer-

ing the composition to a patient using standard means. In some embodiments, the pharmaceutical composition is delivered directly to the site of inflammation using local topical, intravenous, intraperitoneal, and similar methods. Further methodology is discussed below.

In addition to the therapeutic indications, the genes and cells according to this invention can be used in a variety of useful screening and selection methods. In one such method, TNFα non-producer somatic cell variants within a TNFα producing cell population can be selected in vitro by introducing a TNFp-AIG chimeric gene into the TNFα producing cell population. Cells producing TNFα will undergo apoptosis. Cells that do not produce TNFα will survive. Selection of those cell variants possessing the survival phenotype is an easy way to identify TNF-α non-producer cells. Such a selection process may be used to determine expression of genes that act in-trans to regulate activity of the TNF-α promoter, thereby reducing TNF-α production. Such genes are characterized as dominant negative (DN) /dominant suppressive genes in other systems (Behrends S., *et al., J. Biol. Chem.* 1995, 270, 21109-21113; Zhang S., *et al., J. Biol. Chem.*, 1995, 270, 23934-23936; Watowich S.S., *et al., Mol. Cell Biol.*, 1994, 14/6, 3535-3549).

In a further *in vitro* method, a TNFp-AIG chimeric gene according to this invention can be used to identify dominant negative genes responsible for the genesis of a TNFα non-producing cell population. According to this method, a TNFp-AIG chimeric gene according to this invention is introduced into cells that produce TNFα. Barring the presence of a dominant negative gene, those cells should undergo apoptosis upon activation. Therefore, it can be deduced that surviving variants possess a dominant negative gene capable of down-regulating TNFα production. The dominant negative gene can be readily identified by producing a cDNA library and transfecting cell lines (*e.g.*, Jurkat and THP-1). These cells are either stable transfectants of an inducible TNFp-AIG chimeric gene or TNFp-luciferase gene TNFp-AIG transfected cells will be selected for the

survival phenotype following *in vitro* activation; survival phenotype is indicative of the effect of the DN genes. In the cells transfected with TNFp-luciferase gene, reduction in the luciferase activity will be indicative of the DN gene effect. Dominant negative genes identified using this protocol can be used as the future therapeutic agents themselves. Such genes will be the candidates for gene therapy in order to reduce TNFα production.

The methods utilized for gene transfer are grouped into two broad categories:

1. Direct approach : *In situ* transduction of the therapeutic gene into target cells such as synoviocytes using a suitable vector as a carrier for the therapeutic gene. The vector containing therapeutic gene is injected directly into the affected area (*e.g.*, an arthritic joint).

2. Indirect approach : *Ex-vivo* transfection of the therapeutic gene into target cells such as synoviocytes. In this approach, the synovium is removed from joints, synoviocytes are isolated and cultured in vitro. *In vitro* cultured cells are transfected with the therapeutic gene, and genetically modified synoviocytes are transplanted back into the synovium.

For *in vivo* transfer, several vectors have been evaluated for their efficacy in gene delivery (Nita, *et al., Arthritis & Rheumatism*, 1996, 39/5, 820-828). Among the vectors used for gene therapy, the vectors derived from retroviruses are by far the best developed. They are able to insert genetic material in the host genome and produce stable transfectants. These vectors, however are unable to infect non-dividing cells and, since they are inserted in the host genome, the possibility of insertional mutagenesis cannot be ruled out. In comparison, the vectors derived from adenoviruses infect dividing as well as non-dividing cells and deliver DNA episomally. The disadvantage of adenovirus based vectors is that these vectors continue to produce viral proteins in infected cells making them

potentially antigenic. A third type of viral based vectors is derived from Herpes simplex viruses (HSV), which are also capable of infecting dividing as well as non dividing cells.

Among the non-viral vector systems, cationic liposomes and naked plasmid DNA have been evaluated. Liposomes are at the most advanced stage of development, although certain types of cells such as muscle and skin take up, retain and express naked plasmid DNA.

Particle-mediated gene-delivery system is also possible (Rakhmilevich, *et al., PNAS*, 1996, 93, 6291) and is a promising approach.

The following "*in vivo*" gene delivery protocols can be used to deliver the chimeric genes of this invention:

(1) Nita *et al., Arthritis and Rheumatism*, 1996, 39, 820-823

In vivo experiment in rabbits:

Each vector is injected intra-articularly into 1 knee joint. For viral vectors, between 10^8 and 10^9 particles suspended in 0.5 ml balance salt solution are injected per knee.

Liposome-DNA complexes (200 nmoles of DC-Chol complexed with 20 μg of DNA/ml) in 1 ml balance salt solution are injected per knee.

(2) *Methods in Molecular Medicine : Gene Therapy Protocols*, Paul Robbins, ed., 1997, Barr *et al.*, pages 205-212

Adenovirus-based vector delivery to hepatocytes : Rat hepatocytes $1X10^{11}$ PFU in 100 g animal.

In dogs (12-17 kg), portal vein is perfused with about $1.5X10^{11}$ PFU/kg gives 1 adenovirus genome copy per diploid copy of host DNA

In rabbits (2-4kg), $1.5X10^{13}$ virus particles (about $1.5X10^{11}$ PFU) gives 100% hepatocyte transduction; $4X10^{12}$ virus particles give 50-75% transduction.

Yang N-S, *et al.*, 281-296

Gold particle-mediated gene delivery : Transfection of mammalian skin tissue- 0.1, 0.5, 1.0 and 2.5 μg of DNA/mg particle gives linear relationship with transgene expression levels.

Nabel, *et al.*, 297-305

Liposome-mediated gene delivery in humans:

Protocol 1: 15nmol DC-Chol/Dope liposomes combined with 1 μg DNA in 0.7 ml. 0.2 ml of the above mixture is injected into the patient's melanoma nodule. For catheter delivery, 0.6 ml of the solution is delivered into the artery.

Protocol 2: 15nmol DMRIE/Dope liposomes combined with 5 μg DNA in 1.0 ml.

For direct intra-tumor injections, DNA concentrations range from 3 μg complexed with 4.5nM DMRIE/Dope to 300 μg complexed with 450 nM DMRIE/Dope.

(3) Roessler, *et al.* 369-374

Gene transfer to synovium:

A range of doses, 10^9-10^{12} adenovirus particles containing therapeutic gene/joint are used. However, the optimal dose for any particular experimental series needs to be determined empirically, and is dependent on both the properties of the recombinant adenoviral genomic backbone being used as well as the transgene being expressed.

For the indirect approach, a variety of methods are well established, including utilization of cationic lipid or cationic polymer-based transfection and electroporation.

Any of the above-referenced techniques can be altered to suit the particular needs of those of ordinary skill in the art. Such modifications are well within the level of skill possessed by ordinary practitioners and do not require undue experimentation. These obvious variations are within the scope of this invention.

Examples

In order that this invention be more fully understood, the following examples are set forth. These examples are for the purpose of illustrating some preferred embodiments of this invention, and are not to be construed as limiting the scope of this invention in any way.

EXAMPLE 1

Production of TNFp-AIG Constructs

In order to construct chimeric AIG driven by the enhancer cis elements of the TNF promoter, either in a single or multiple copies of the same region or various regions, identification of the regions of interest responsible for optimal inducible expression of the reporter gene is performed.

Selection of the TNF-α promoter elements for constructing a chimeric gene. The regions of the TNF-α promoter are amplified by polymerase chain reaction (PCR) using primers encompassing various deletion constructs of the TNFα promoter (Figure 3). The regions identified by other investigators in various other cellular systems are used as reference (Rhoades, *et al., J. Biol. Chem.*, 1992, 267, 22102-22107; Leitman, *et al., Mol. Cell Biol.*, 1992, 12, 1352-1356; Pauli U., *Crit. Reviews Eukaryotic Gene Expression*, 1994, 4, 323-344). The PCR-amplified genes are then cloned upstream of a reporter gene, such as luciferase, in a commercially available promoterless vector. These

constructs are tested for their constitutive and inducible expression in various cell lines such as Jurkat (T lymphoblastoid), U973 (myelomonocytic), THP-1 (monocytic), fibroblasts and in vitro cultured human synoviocytes. Identification of the regions responsible for inducible expression of the reporter gene is primarily based on the results obtained using two TNFα-producing cell lines, viz Jurkat (following stimulation with PMA) and THP-1 (following stimulation with LPS) (Figure 4 a and b). These cells are transiently transfected by using well established methods and commercially available reagents, *e.g.*, DEAE dextran and Superfect. The cis-elements of the TNFα promoter that are responsible for inducible expression of the reporter gene are then used for constructing TNFp-AIG chimeric genes.

Construction of TNFp-AIG chimeric genes. Of the apoptosis inducing genes described herein, the following genes are preferred:

i) cysteine protease - CPP32 (also known as Yama, apopain or Caspase 3) and

ii) Cysteine protease -Tx/Ty (Caspase 4/Caspase 5)

The AIGs are used as "prodomain-deleted"truncations in order to potentially augment autocatalysis of Caspases. This is essential for conversion of inactive Caspase to active form.

Prodomain-deleted CPP32 is amplified using primers corresponding to codons 29-36 and 271-278 (278 is a stop codon). The truncated form of CPP32 is referred to as "αCPP32" or "AIG.1" herein.

For PCR amplification for prodomain-deleted Ty, primers corresponding to the sequences in the Ty gene are synthesized. All Caspases discovered so far have homology to the other members of the Caspase family. The 3' primer corresponding to the codons 359-365 (codon 365 is a stop codon) shares 100% sequence homology to the codons 372-378 (codon 378 is a stop codon) in the Tx gene. However, the 5' primer corresponding to codons 81-87 in the Ty gene does not share 100% homology with the corresponding region in the Tx gene (Tx codons 94-100). Residue 87 (Alanine) in the Ty gene differs from residue 100 (Glycine) in the Tx gene. The PCR amplified product generated from cDNA prepared from activated human peripheral blood lymphocytes possesses the

sequence of Tx, due to apparent abundance of Tx transcripts. Therefore, the truncated form of the AIG generated using synthetics oligonucleotide primers corresponding to the sequences in Ty, indeed matches sequences in Tx , albeit flanked by Ty sequences of the primers. The Ty sequences of the primers used also match with the sequence of Tx, except for one codon. Thus the gene used in this invention matches truncated Tx gene with residue G100 toA change. This gene is referred to as "ΔTy/x" or "AIG.2" herein.

AIG.1 and AIG.2 are inserted downstream of the TNFα promoter by replacing the luciferase reporter gene in deletion constructs (-120, -706 and -1005) of the TNFα promoter (Figure 5). These constructs are tested for the induction of apoptosis following stimulation of transiently-infected Jurkat and THP-1 cells (Figures 6 a, b, and c).

Constuction of TNFα superpromoter-AIG chimeric genes. Two broad preferred regions, *viz.*, ER1(-1005 to -905) (SEQ. ID 10) and ER2(-706 to -517) (SEQ ID NO: 11) of the TNFα promoter, containing elements responsible for inducible expression of the reporter gene described above (Figure 4a and 4b) are PCR amplified and are ligated upstream of the minimal native promoter (-120 through TSS, SEQ ID NO: 3), either as a single copy or multiple copies. Two more regions (-234 to -120) and (-234 to -65) of the TNFα promoter is also identified as a potential enhancer region 3 (ER3) and enhancer region 4 (ER4), respectively, which can be employed in the chimeric constructs using the strategies described below. The super promoter contains multiple (2-10) cassettes of the above mentioned regions containing inducible promoter elements (Figure 7). This is achieved by PCR amplifying the regions of interest using primers synthesized with restriction sites inserted at the 5' end of each of the primers. These unique restriction sites flank the amplified gene product of interest. Preferably, PCR amplified AIG is cloned downstream of the TNF-(super promoter, replacing the luciferase reporter gene in the original construct as described (Figure 5) for the native TNFα promoter.

The schemes for construction of a TNFα superpromoter and the linker sequences representing unique restriction sites (these restriction sites are absent in the selected elements of the TNF-α promoter and the AIG in question) for efficient directional insertion is outlined below and depicted in Figure 8:

Scheme 1:

STEP 1: Insertion of the TNF-α minimal promoter (-120 to TSS) into the pGL3 basic (promoterless) luciferase vector (Promega):

The elements of pGL3 basic vectors that are used for construction of the chimeric gene TNFp-AIG are shown below.

_____KpnI.SacI.MluI.NheI.SmaI.XhoI.BglII.HindIII. [luciferase].XbaI _____

The minimal promoter is PCR amplified using primers containing XhoI and BglII.HindIII sites, so that XhoI is at the 5'end and BglII.HindIII sites are at the 3' end of the amplified product. This fragment is inserted into the polylinker of the pGL3 basic vector using these same restriction sites. This construct is referred to as "Construct A1" and is as follows:

___KpnI.SacI.MluI.NheISmaIXhoI.(-120 to TSSBglII).HindIII.[luciferase].XbaI___

STEP 2: The enhancer fragment (ER1 or ER2) is PCR amplified using the primer containing several restriction sites. The resulting fragment will have restriction sites KpnI.AatII.BssHII at the 5' end and NsiI.SpeI.MluI at the 3'end as follows: 5' KpnI.AatII.BssHII.(ER1 or ER2).NsiI.SpeI.MluI 3". The fragment is inserted into the "Construct A1" generated in STEP 1 using KpnI and MluI restriction sites. This construct is referred to as "Construct B1" and is as follows:

_____KpnI.AatII.BssHII.(ER1 or ER2).NsiI.SpeI.MluI.NheI.SmaI.XhoI(-120 to TSS BglII).HindIII. [luciferase].XbaI_____

STEP 3: The TNFα enhancer fragment (ER1 or ER2)) is amplified using the primers containing restriction sites AatII and BssHII to generate the PCR product as follows:

5' AatII.(ER1 or ER2).BssHII 3'. This fragment is cloned into the "Construct B1" using these same restriction sites. This construct is referred to as "Construct C1" and is as follows:

_____KpnI.AatII.(ER1 or ER2).BssHII.(ER1 or ER2).NsiI.SpeI.MluI.NheI SmaI.XhoI(-120 to TSS BglII).HindIII.[luciferase].XbaI_____

STEP 4: The TNFα enhancer fragment (ER1 or ER2) is amplified using the primers containing restriction sites NsiI and SpeI to generate the PCR product as follows:

5' NsiI.(ER1 or ER2).SpeI 3'. This fragment will be cloned into the "Construct C1" using these same restriction sites. This construct is referred to as "Construct D1" and is as follows:

_____KpnI.AatII.(ER1 or ER2).BssHII.(ER1 or ER2).NsiI.(ER1 or ER2).SpeI.MluI.NheI SmaI.XhoI(-120 to TSSBglII).HindIII.[luciferase].XbaI

STEP 5: AIG.1 or AIG.2 (preferred but not limited to AIG.1 and AIG.2; any AIG from the list can be used) coding regions are PCR-amplified using the primers containing BglII and XbaI restriction sites generating the fragment as follows: 5' BglII.(AIG.1 or AIG.2).XbaI 3". This fragment is inserted into the "Construct D1" using these same restriction sites. The resulting construct is referred to as "Construct E1" and is as follows:

_____KpnI.AatII.(ER1 or ER2).BssHII.(ER1 or ER2).NsiI.(ER1 or ER2).SpeI.MluI.NheI.SmaI.XhoI(-120 to TSS.BglII)[AIG.1 or AIG.2].XbaI_____

Alternatively scheme 2 is followed:

Scheme 2:

STEP 1: Same as in scheme I giving rise to "Construct A1", which is as follows:

KpnI.SacI.MluI.NheI.SmaI.XhoI.(-120 to TSS BglII).HindIII.[luciferase].XbaI

STEP 2: Insertion of additional MCS.

Two complementary oligonucleotides (5' phosphorylated) providing _____NheI.SacII.EcorV.AflII.AatII.AvrII.SpeI.PvuII.XhoI_____are synthesized using commercial sources. These oligonucleotides are annealed and then cloned into NheI and XhoI sites of the "Construct A1". The resulting construct referred to as "Construct B2" and it is as follows:

_____KpnI.SacI.MluI.NheI. SacII.EcorV.AflII.AatII.AvrII.SpeI.PvuII.XhoI.(-120 to TSS BglII).HindIII.[luciferase].XbaI_____

STEP 3: The TNF-α enhancer fragment (ER1 or ER2) is amplified using the primers containing restriction sites SpeI.PvuII at the 5' end, and XhoI at the 3' end to generate the PCR product as follows: 5' SpeI.PvuII.(ER1 or ER2) . XhoI 3'. This fragment is cloned into the "Construct B2" using SpeI and XhoI restriction sites. This construct is referred to as "Construct C2" and is as follows:

_____KpnI.SacI.MluI.NheI. SacII.EcorV.AflII.AatII.AvrII.SpeI.PvuII.(ER1 or ER2) XhoI.(-120 to TSS BglII).HindIII.[luciferase].XbaI_____

STEP 4: The TNFα enhancer fragment (ER1 or ER2) is amplified using the primers containing restriction sites AvrII. SpeI at the 5' end, and and PvuII at the 3' end to generate the PCR product as follows: 5' AvrII.SpeI.(ER1 or ER2).PvuII 3'. This fragment is cloned into the "Construct C2" using AvrII and

PvuII restriction sites. This construct is referred to as "Construct D2" and is as follows:

_____KpnI.SacI.MluI.NheI. SacII.EcorV.AflII.AatII.AvrII.SpeI. (ER1 or ER2) PvuII. (ER1 or ER2) XhoI.(-120 to TSS BglII).HindIII.[luciferase].XbaI_____

Thus, using this strategy at least seven copies of the enhancer regions (ER1, ER2 or ER3, individually or in combination), one at a time, can be added by using one more restriction site upstream of the previous one in PCR amplification of the enhancer regions of choice.

Once the desired number of copies of the enhancer regions are added, AIG is inserted downstream of the superpromoter as described in the STEP 5 of the scheme 1.

The inducible expression of the chimeric TNFp-AIG gene is tested by transient transfection of the cell lines mentioned above. The expression of TNFp-AIG gene is measured by detecting apoptosis of transfected cells, assessing AIG expressed proteins in Western blots using commercially available antibodies and assessing protease activity using commercially available, well documented specific synthetic tetrapeptide substrate.

The inducible expression of the chimeric TNFp-FasL gene is tested by transient transfection of the same cell lines. The cell surface expression of FasL by the transfected cells is quantitated using anti-FasL antibody binding as detected by indirect immunofluorescence and by measuring induction of apoptosis of Fas positive cells.

Regulation of the TNFp-driven expression of a reporter gene. The 3' untranslated region of the TNFα gene plays an important role in reulation of the TNFα biosynthesis. It is involved in translational expression of the TNFα gene in

normal, non-activated states. Importantly, these elements allow de-repression to occur when TNFα-producing cells are activated by external stimuli (Han, J., *et al., J. Immunology*, 1991, 146, 1843-1848; Crawford, F.K., *et al., J. Biol. Chem.*, 1996, 271, 22383-22390).

Genetic constructs are made in which the entire 3' untranslated region (SEQ ID NO: 13) is inserted downstream of the luciferase gene driven by deletion fragments, *viz.*, -120, -706 and -1005 of the TNFα promoter. The results of the transient expression of these constructs are summarized in Figure 9.

EXAMPLE 2

Testing Protocols

In Vitro Methods:

Luciferase assay: Luciferase activity is determined using commercially available reagents (Promega).

AIG.1 and AIG.2 gene expression:

a) Western blots of the transfected cell lysates are developed using anti-CPP32 antibody as well as anti-PRAP antibody. Anti-PRAP antibody detects both hydrolyzed as well as non-hydrolyzed products of PRAP as an enzymatic action of CPP32.

b) CPP32 enzyme assay : This assay detects enzymatic reaction of CPP32 and breakdown of colorimetric or fluorogenic substrate. Commercially available (Clonotech, Pharmingen) kit ise used for this assay.

c) Apoptosis of transfected cells: Apoptosis of transfected cells due to AIG.1 and AIG.2 is determined by staining nuclei by propidium iodide (Krishan, A., J. Cell Biol., 66, 1994, 188-193) and by commercially available Cell Death Elisa kit (Boehringer Mannheim).

Animal Models

Rabbit model of IL-1-induced arthritis (Pettipher E. R., *et al., Proc. Natl. Acad. Sci.*, 1986, 83, 8749-8753): Il-1 is injected into the knee joints of New Zealand White rabbits. Intra-articular injection of IL-1 causes dose-dependent infiltration of leukocytes into the joint space and loss of proteoglycan from the articular cartilage.

Antigen-Induced arthritis : Intra-articular injection of antigen (ovalbumin) into knee joints induces leukocyte accumulation and cartilage degradation that closely resembles rheumatoid arthritis in humans. The joint swelling following the injection was sustained for 14 days.

Scid mice-human synoviocytes model (Houri J.M., *et al. Current Opinions in Rheumatol.*, 1995, 7, 201-205; Sack U., *et al., J. Autoimmunity*, 1995, 9, 51-58; Geiler T., *et al. Arthritis & Rheumatism*, 1994, 37, 1664-1671): These are recently developed models for arthritis in which fresh synovial tissue from RA patients is implanted with normal human cartilage into scid mice either subcutaneously, under the renal capsule (Geiler T., *et al., Arthritis & Rheumatism*, 1994, 37, 1664-1671), or into knee joints (Sack U., *et al., J. Autoimmunity*, 1995, 9, 51-58). The implants grow with arthritis-like characteristics, including formation of pannus tissue of high cellular density, bone and cartilage erosion, development of multinuclear giant cells, and invasion of cartilage by synovial fibroblasts.

Indirect Method: Synoviocytes are transfected *in vitro* with the therapeutic gene and transplanted back in rabbits. Arthritis is induced in these rabbits by injecting IL-1 and expression of the therapeutic gene following activation is assessed. Activation-induced expression of the chimeric gene induces apoptosis in transplanted cells.

Direct Method: Intra-articular injection of the chimeric genes. Any of the gene delivery methods described above, including naked plasmid DNA,

cationic liposome-mediated delivery can be used. For use of viral vector-based delivery, chimeric genes are cloned in suitable vectors. The vectors are then modified by deleting eukaryotic promoter present in these vectors. Intra-articular injection of the therapeutic genes inserted in appropriate vectors can then be done to assess therapeutic as well as prophylactic efficacy.

EXAMPLE 3

Selection of TNF-α Non-Producer Somatic Cell Variants

Cells (THP-1, Jurkat) are stably transfected *in vitro* with TNFp-AIG chimeric gene. After several cycles of stimulation, which induces apoptosis in the cells expressing the TNFp-AIG gene, surviving cells are then collected. A cDNA library from these cells is constructed, which is used for functional cloning (Legerski R and Peterson C., *Nature*, 1992, 359, 70-73; Jaattela M., *et al.*, *Oncogene*, 1995, 10, 2297-2305).

EXAMPLE 4

Identification and Characterization of Dominant Negative (DN) Genes

THP-1 and Jurkat cells stably transfected with TNFp-AIG are subjected to repeated cycles of stimulation to activate expression of TNFp-AIG. The cells, which do not express negative regulatory genes, undergo apoptosis, whereas those expressing dominant negative genes survive. In these surviving cells DN gene products act in-trans with the TNFα promoter, thereby inhibiting its activations to transcribe AIG, ultimately resulting in survival phenotype. cDNA library is constructed using polyadenylated mRNA from these cells. The DN genes which rescue TNFp-AIG-transfected THP-1 or Jurkat cells from apoptosis are identified by functional cloning as described for other genes (Legerski R. and Peterson C., *Nature*, 1992, 359, 70-73; Jaattela M., *et al.*, *Oncogene*, 1995, 10, 2297-2305).

The above description is for the purpose of teaching the person of ordinary skill in the art how to practice the present invention, and it is not intended to detail all those obvious modifications and variations of it which will become apparent to the skilled worker upon reading the description. It is intended, however, that all such obvious modifications and variations be included within the scope of the present invention, which is defined by the following claims. The claims are intended to cover the claimed components and steps in any sequence which is effective to meet the objectives there intended, unless the context specifically indicates the contrary.

The papers cited herein are expressly incorporated in their entireties by reference.

SEQUENCE LISTING

(1) GENERAL INFORMATION:

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(ii) TITLE OF INVENTION: Self-Regulated Apoptosis of Inflammatory Cells by Gene Therapy

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(B) TELEFAX NUMBER: 203-791-6183

(2) INFORMATION FOR SEQ ID NO: 1:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 1178
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE:
(A) DESCRIPTION: DNA

(ix) FEATURE:
(A) NAME/KEY: reference human TNFα promoter

(x) PUBLICATION INFORMATION:
(A) AUTHORS: Takashiba, S., *et al.*
(C) JOURNAL: Gene
(D) VOLUME: 131
(e) PAGES: 307-308

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 1:

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TCCTGAGGCC TCAAGCTGCC ACCAAGCCCC CAGCTCCTTC TCCCCGCAGA 550
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TTCTCTCCCT CAAGGACTCA GCTTTCTGAA GCCCCTCCCA GTTCTAGTTC 650
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GGGGTTCAGC CTCCAGGGTC CTACACACAA ATCAGTCAGT GGCCCAGAAG 800
ACCCCCCTCG GAATCGGAGC AGGGAGGATG GGGAGTGTGA GGGGTATCCT 850
TGATGCTTGT GTGTCCCCAA CTTTCCAAAT NCCCGCCCCC GCGATGGAGA 900
AGAAACCGAG ACAGAAGGTG CAGGGCCCAC TACCGCTTCC TCCAGATGAG 950
CTTATGGGTT TCTCCACCAA GGAAGTTTTC CGCTGGTTGA ATGATTCTTT 1000
CCCCGCCCTC CTCTCGCCCC AGGGACATAT AAAGGCAGTT GTTGGCACAC 1050
CCAGCCAGCA GACGCTCCCT CAGCAAGGAC AGCAGAGGAC CAGCTAAGAG 1100
GGAGAGAAGC AACTGCAGAC CCCCCCTGAA AACAACCCTC AGACGCCACA 1150
TCCCCTGACA AGCTGCCAGG CAGGTTCT 1178

(3) INFORMATION FOR SEQ ID NO: 2:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 1096
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE:
(A) DESCRIPTION: DNA

(ix) FEATURE:
(A) NAME/KEY: human TNFα promoter gene

(x) PUBLICATION INFORMATION:
(A) AUTHORS: Takashiba, S., *et al.*
(C) JOURNAL: Gene
(D) VOLUME: 131
(e) PAGES: 307-308

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 2:

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AGATGTGACC ACAGCAATGG GTAGGAGAAT GTCCAGGGCT ATGGAAGTCG     200
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GGAGTGAAAG AGCCTCCAGG ACCTCCAGGT ATGGAATACA GGGGACGTTT     300
AAGAAGATAT GGCCACACAC TGGGGCCCTG AGAAGTGAGA GCTTCATGAA     350
AAAAATCAGG GACCCCAGAG TTCCTTGGAA GCCAAGACTG AAACCAGCAT     400
TATGAGTCTC CGGGTCAGAA TGAAAGAAGA AGGCCTGCCC CAGTGGGGTC     450
TGTGAATTCC CGGGGGTGAT TTCACTCCCC GGGGCTGTCC CAGGCTTGTC     500
CCTGCTACCC CCACCCAGCC TTTCCTGAGG CCTCAAGCCT GCCACCAAGC     550
CCCCAGCTCC TTCTCCCCGC AGGGACCCAA ACACAGGCCT CAGGACTCAA     600
CACAGCTTTT CCCTCCAACC CCGTTTTCTC TCCCTCAAGG ACTCAGCTTT     650
CTGAAGCCCC TCCCAGTTCT AGTTCTATCT TTTTCCTGCA TCCTGTCTGG     700
AAGTTAGAAG GAAACAGACC ACAGACCTGG TCCCCAAAAG AAATGGAGGC     750
AATAGGTTTT GAGGGGCATG GGGACGGGGT TCAGCCTCCA GGGTCCTACA     800
CACAAATCAG TCAGTGGCCC AGAAGACCCC CCTCGGAATC GGAGCAGGGA     850
GGATGGGGAG TGTGAGGGGT ATCCTTGATG CTTGTGTGTC CCCAACTTTC     900
CAAATCCCCG CCCCCGCGAT GGAGAAGAAA CCGAGACAGA AGGTGCAGGG     950
CCCACTACCG CTTCCTCCAG ATGAGCTCAT GGGTTTCTCC ACCAAGGAAG    1000
TTTTCCGCTG GTTGAATGAT TCTTTCCCCG CCCTCCTCTC GCCCCAGGGA    1050
CATATAAAGG CAGTTGTTGG CACACCCAGC CAGCAGACGC TCCCTC        1096
```

(4) INFORMATION FOR SEQ ID NO: 3:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 139
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE:
(A) DESCRIPTION: DNA

(ix) FEATURE:
(A) NAME/KEY: native minimal TNFα promoter

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 3:

```
CCGCTTCCTC CAGATGAGCT CATGGGTTTC TCCACCAAGG AAGTTTTCCG      50
CTGGTTGAAT GATTCTTTCC CCGCCCTCCT CTCGCCCCAG GGACATATAA     100
AGGCAGTTGT ATGGCACACC CGCCAGCAGA CGCTCCCTC                 139
```

(5) INFORMATION FOR SEQ ID NO: 4:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 904
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE:
(A) DESCRIPTION: DNA

(ix) FEATURE:
(A) NAME/KEY: chimeric gene TNFp120 AIG.1
(D) OTHER INFORMATION: residues 1 to 139 comprise the promoter sequence; residues 140 to 151, the linker sequence, and the remaining residues comprise the AIG.1 sequence

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 4:

CCGCTTCCTC CAGATGAGCT CATGGGTTTC TCCACCAAGG AAGTTTTCCG 50
CTGGTTGAAT GATTCTTTCC CCGCCCTCCT CTCGCCCCAG GGACATATAA 100
AGGCAGTTGT TGGCACACCC AGCCAGCAGA CGCTCCCTCA GCAGATCCAC 150
CATGTCTGGA ATATCCCTGG ACAACAGTTA TAAAATGGAT TATCCTGAGA 200
TGGGTTTATG TATAATAATT AATAATAAGA ATTTTCATAA AAGCACTGGA 250
ATGACATCTC GGTCTGGTAC AGATGTCGAT GCAGCAAACC TCAGGGAAAC 300
ATTCAGAAAC TTGAAATATG AAGTCAGGAA TAAAAATGAT CTTACACGTG 350
AAGAAATTGT GGAATTGATG CGTGATGTTT CTAAAGAAGA TCACAGCAAA 400
AGGAGCAGTT TTGTTTGTGT GCTTCTGAGC CATGGTGAAG AAGGAATAAT 450
TTTTGGAACA AATGGACCTG TTGACCTGAA AAAAATAACA AACTTTTTCA 500
GAGGGGATCG TTGTAGAAGT CTAACTGGAA AACCCAAACT TTTCATTATT 550
CAGGCCTGCC GTGGTACAGA ACTGGACTGT GGCATTGAGA CAGACAGTGG 600
TGTTGATGAT GACATGGCGT GTCATAAAAT ACCAGTGGAG GCCGACTTCT 650
TGTATGCATA CTCCACAGCA CCTGGTTATT ATTCTTGGCG AAATTCAAAG 700
GATGGCTCCT GGTTCATCCA GTCGCTTTGT GCCATGCTGA AACAGTATGC 750
CGACAAGCTT GAATTTATGC ACATTCTTAC CCGGGCTAAC CGAAAGGTGG 800
CAACAGAATT TGAGTCCTTT TCCTTTGACG CTACTTTTCA TGCAAAGAAA 850
CAGATTCCAT GTATTGTTTC CATGCTCACA AAAGAACTCT ATTTTTATCA 900
CTAA 904

(6) INFORMATION FOR SEQ ID NO: 5:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 1490
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE:
(A) DESCRIPTION: DNA

(ix) FEATURE:
(A) NAME/KEY: chimeric gene TNFp706 AIG.1
(D) OTHER INFORMATION: residues 1 to 724 comprise the

promoter sequence; residues 725 to 736, the linker sequence, and the remaining residues comprise the AIG.1 sequence

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 5:

```
TCCTTGGAAG CCAAGACTGA AACCAGCATT ATGAGTCTCC GGGTCAGAAT      50
GAAAGAAGAA GGCCTGCCCC AGTGGGGTCT GTGAATTCCC GGGGGTGATT     100
TCACTCCCCG GGGCTGTCCC AGGCTTGTCC CTGCTACCCC CACCCAGCCT     150
TTCCTGAGGC TCAAGCCTGC CACCAAGCCC CCAGCTCCTT CTCCCCGCAG     200
GGACCCAAAC ACAGGCCTCA GGACTCAACA CAGCTTTTCC CTCCAACCCC     250
GTTTTCTCTC CCTCAAGGAC TCAGCTTTCT GAAGCCCCTC CCAGTTCTAG     300
TTCTATCTTT TTCCTGCATC CTGTCTGGAA GTTAGAAGGA AACAGACCAC     350
AGACCTGGTC CCCAAAAGAA ATGGAGGCAA TAGGTTTTGA GGGGCATGGG     400
GACGGGGTTC AGCCTCCAGG GTCCTACACA CAAATCAGTC AGTGGCCCAG     450
AAGACCCCCC TCGGAATCGG AGCAGGGAGG ATGGGGAGTG TGAGGGGTAT     500
CCTTGATGCT TGTGTGTCCC CAACTTTCCA AATCCCCGCC CCCGCGATGG     550
AGAAGAAACC GAGACAGAAG GTGCAGGGCC CACTACCGCT TCCTCCAGAT     600
GAGCTCATGG GTTTCTCCAC CAAGGAAGTT TTCCGCTGGT TGAATGATTC     650
TTTCCCCGCC CTCCTCTCGC CCCAGGGACA TATAAAGGCA GTTGTTGGCA     700
CACCCAGCCA GCAGACGCTC CCTCAGCAGA TCCACCATGT CTGGAATATC     750
CCTGGACAAC AGTTATAAAA TGGATTATCC TGAGATGGGT TTATGTATAA     800
TAATTAATAA TAAGAATTTT CATAAAAGCA CTGGAATGAC ATCTCGGTCT     850
GGTACAGATG TCGATGCAGC AAACCTCAGG GAAACATTCA GAAACTTGAA     900
ATATGAAGTC AGGAATAAAA ATGATCTTAC ACGTGAAGAA ATTGTGGAAT     950
TGATGCGTGA TGTTTCTAAA GAAGATCACA GCAAAAGGAG CAGTTTTGTT    1000
TGTGTGCTTC TGAGCCATGG TGAAGAAGGA ATAATTTTTG GAACAAATGG    1050
ACCTGTTGAC CTGAAAAAAA TAACAAACTT TTTCAGAGGG GATCGTTGTA    1100
GAAGTCTAAC TGGAAAACCC AAACTTTTCA TTATTCAGGC CTGCCGTGGT    1150
ACAGAACTGG ACTGTGGCAT TGAGACAGAC AGTGGTGTTG ATGATGACAT    1200
GGCGTGTCAT AAAATACCAG TGGAGGCCGA CTTCTTGTAT GCATACTCCA    1250
CAGCACCTGG TTATTATTCT TGGCGAAATT CAAAGGATGG CTCCTGGTTC    1300
ATCCAGTCGC TTTGTGCCAT TGCTGAAACA GTATGCCGAC AAGCTTGAAT    1350
TTATGCACAT TCTTACCCGG GCTAACCGAA AGGTGGCAAC AGAATTTGAG    1400
TCCTTTTCCT TTGACGCTAC TTTTCATGCA AAGAAACAGA TTCCATGTAT    1450
TGTTTCCATG CTCACAAAAG AACTCTATTT TTATCACTAA               1490
```

(7) INFORMATION FOR SEQ ID NO: 6:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 1789
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE:
(A) DESCRIPTION: DNA

(ix) FEATURE:
(A) NAME/KEY: chimeric gene TNFp1005 AIG.1
(D) OTHER INFORMATION: residues 1 to 1023 comprise the promoter sequence; residues 1024 to 1036, the linker sequence, and the remaining residues comprise the AIG.1 sequence

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 6:

GGCGGGGGTC AGGGAGCTCC TGGGAGATAT GGCCACATGT AGCGGCTCTG 50
AGGAATGGGT TACAGGAGAC CTCTGGGGAG ATGTGACCAC AGCAATGGGT 100
AGGAGAATGT CCAGGGCTAT GGAAGTCGAG TATGGGGACC CCCCCTTAAC 150
GAAGACAGGG CCATGTAGAG GGCCCCAGGG AGTGAAAGAG CCTCCAGGAC 200
CTCCAGGTAT GGAATACAGG GGACGTTTAA GAAGATATGG CCACACACTG 250
GGGCCCTGAG AAGTGAGAGC TTCATGAAAA AAATCAGGGA CCCCAGAGTT 300
CCTTGGAAGC CAAGACTGAA ACCAGCATTA TGAGTCTCCG GGTCAGAATG 350
AAAGAAGAAG GCCTGCCCCA GTGGGGTCTG TGAATTCCCG GGGGTGATTT 400
CACTCCCCGG GGCTGTCCCA GGCTTGTCCC TGCTACCCCC ACCCAGCCTT 450
TCCTGAGGCC TCAAGCCTGC CACCAAGCCC CCAGCTCCTT CTCCCCGCAG 500
GGACCCAAAC ACAGGCCTCA GGACTCAACA CAGCTTTTCC CTCCAACCCC 550
GTTTTCTCTC CCTCAAGGAC TCAGCTTTCT GAAGCCCCTC CCAGTTCTAG 600
TTCTATCTTT TTCCTGCATC CTGTCTGGAA GTTAGAAGGA AACAGACCAC 650
AGACCTGGTC CCCAAAAGAA ATGGAGGCAA TAGGTTTTGA GGGGCATGGG 700
GACGGGGTTC AGCCTCCAGG GTCCTACACA CAAATCAGTC AGTGGCCCAG 750
AAGACCCCCC TCGGAATCGG AGCAGGGAGG ATGGGGAGTG TGAGGGGTAT 800
CCTTGATGCT TGTGTGTCCC CAACTTTCCA AATCCCCGCC CCCGCGATGG 850
AGAAGAAACC GAGACAGAAG GTGCAGGGCC CACTACCGCT TCCTCCAGAT 900
GAGCTCATGG GTTTCTCCAC CAAGGAAGTT TTCCGCTGGT TGAATGATTC 950
TTTCCCCGCC CTCCTCTCGC CCCAGGGACA TATAAAGGCA GTTGTTGGCA 1000
CACCCAGCCA GCAGACGCTC CCTCAGCAGA TCCACCATGT CTGGAATATC 1050
CCTGGACAAC AGTTATAAAA TGGATTATCC TGAGATGGGT TTATGTATAA 1100
TAATTAATAA TAAGAATTTT CATAAAAGCA CTGGAATGAC ATCTCGGTCT 1150
GGTACAGATG TCGATGCAGC AAACCTCAGG GAAACATTCA GAAACTTGAA 1200
ATATGAAGTC AGGAATAAAA ATGATCTTAC ACGTGAAGAA ATTGTGGAAT 1250
TGATGCGTGA TGTTTCTAAA GAAGATCACA GCAAAAGGAG CAGTTTTGTT 1300
TGTGTGCTTC TGAGCCATGG TGAAGAAGGA ATAATTTTTG GAACAAATGG 1350
ACCTGTTGAC CTGAAAAAAA TAACAAACTT TTTCAGAGGG GATCGTTGTA 1400
GAAGTCTAAC TGGAAAACCC AAACTTTTCA TTATTCAGGC CTGCCGTGGT 1450
ACAGAACTGG ACTGTGGCAT TGAGACAGAC AGTGGTGTTG ATGATGACAT 1500
GGCGTGTCAT AAAATACCAG TGGAGGCCGA CTTCTTGTAT GCATACTCCA 1550
CAGCACCTGG TTATTATTCT TGGCGAAATT CAAAGGATGG CTCCTGGTTC 1600
ATCCAGTCGC TTTGTGCCAT GCTGAAACAG TATGCCGACA AGCTTGAATT 1650
TATGCACATT CTTACCCGGG CTAACCGAAA GGTGGCAACA GAATTTGAGT 1700
CCTTTTCCTT TGACGCTACT TTTCATGCAA AGAAACAGAT TCCATGTATT 1750
GTTTCCATGC TCACAAAAGA ACTCTATTTT TATCACTAA 1789

(8) INFORMATION FOR SEQ ID NO: 7:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 1008
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE:
(A) DESCRIPTION: DNA

(ix) FEATURE:
(A) NAME/KEY: chimeric gene TNFp120 AIG.2
(D) OTHER INFORMATION: residues 1 to 138 comprise the

promoter sequence; residues 139 to 150, the linker sequence, and the remaining residues comprise the AIG.2 sequence

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 7:

```
CCGCTTCCTC CAGATGAGCT CATGGGTTTC TCCACCAAGG AAGTTTTCCG     50
CTGGTTGAAT GATTCTTTCC CCGCCCTCCT CTCGCCCCAG GGACATATAA    100
AGGCAGTTGT TGGCACACCC AGCCAGCAGA GCTCCCTCAG CAGATCCACC    150
ATGGCTGGAC CACCTGAGTC AGCAGAATCT ACAGATGCCC TCAAGCTTTG    200
TCCTCATGAA GAATTCCTGA GACTATGTAA AGAAAGAGCT GAAGAGATCT    250
ACCCAATAAA GGAGAGAAAC AACCGCACAC GCCTGGCTCT CATCATATGC    300
AATACAGAGT TTGACCATCT GCCTCCGAGG AATGGAGCTG ACTTTGACAT    350
CACAGGGATG AAGGAGCTAC TTGAGGGTCT GGACTATAGT GTAGATGTAG    400
AAGAGAATCT GACAGCCAGG GATATGGAGT CAGCGCTGAG GGCATTTGCT    450
ACCAGACCAG AGCACAAGTC CTCTGACAGC ACATTCTTGG TACTCATGTC    500
TCATGGCATC CTGGAGGGAA TCTGCGGAAC TGTGCATGAT GAGAAAAAAC    550
CAGATGTGCT GCTTTATGAC ACCATCTTCC AGATATTCAA CAACCGCAAC    600
TGCCTCAGTC TGAAGGACAA ACCCAAGGTC ATCATTGTCC AGGCCTGCAG    650
AGGTGCAAAC CGTGGGGAAC TGTGGGTCAG AGACTCTCCA GCATCCTTGG    700
AAGTGGCCTC TTCACAGTCA TCTGAGAACC TGGAGGAAGA TGCTGTTTAC    750
AAGACCCACG TGGAGAAGGA CTTCATTGCT TTCTGCTCTT CAACGCCACA    800
CAACGTGTCC TGGAGAGACA GCACAATGGG CTCTATCTTC ATCACACAAC    850
TCATCACATG CTTCCAGAAA TATTCTTGGT GCTGCCACCT AGAGGAAGTA    900
TTTCGGAAGG TACAGCAATC ATTTGAAACT CCAAGGGCCA AAGCTCAAAT    950
GCCCACCATA GAACGACTGT CCATGACAAG ATATTTCTAC CTCTTTCCTG   1000
GCAATTGA                                                 1008
```

(9) INFORMATION FOR SEQ ID NO: 8:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 1587
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE:
(A) DESCRIPTION: DNA

(ix) FEATURE:
(A) NAME/KEY: chimeric gene TNFp706 AIG.2
(D) OTHER INFORMATION: residues 1 to 724 comprise the promoter sequence; residues 725 to 736, the linker sequence, and the remaining residues comprise the AIG.2 sequence

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 8:

```
TCCTTGGAAG CCAAGACTGA AACCAGCATT ATGAGTCTCC GGGTCAGAAT     50
GAAAGAAGAA GGCCTGCCCC AGTGGGGTCT GTGAATTCCC GGGGGTGATT    100
TCACTCCCCG GGGCTGTCCC AGGCTTGTCC CTGCTACCCC CACCCAGCCT    150
TTCCTGAGGC CTCAAGCCTG CCACCAAGCC CCCAGCTCCT TCTCCCCGCA    200
GGGACCCAAA CACAGGCCTC AGGACTCAAC ACAGCTTTTC CCTCCAACCC    250
CGTTTTCTCT CCCTCAAGGA CTCAGCTTTC TGAAGCCCCT CCCAGTTCTA    300
GTTCTATCTT TTTCCTGCAT CCTGTCTGGA AGTTAGAAGG AAACAGACCA    350
```

```
CAGACCTGGT CCCCAAAAGA AATGGAGGCA ATAGGTTTTG AGGGGCATGG   400
GGACGGGGTT CAGCCTCCAG GGTCCTACAC ACAAATCAGT CAGTGGCCCA   450
AAGACCCCCC TCGGAATCGG AGCAGGGAGG ATGGGGAGTG TGAGGGGTAT   500
CCTTGATGCT TGTGTGTCCC CAACTTTCCA AATCCCCGCC CCCGCGATGG   550
AGAAGAAACC GAGACAGAAG GTGCAGGGCC CACTACCGCT TCCTCCAGAT   600
GAGCTCATGG GTTTCTCCAC CAAGGAAGTT TTCCGCTGGT TGAATGATTC   650
TTTCCCCGCC CTCCTCTCGC CCCAGGGACA TATAAAGGCA GTTGTTGGCA   700
CACCCAGCCA GCAGACGCTC CCTCAGCAGA TCCACCATGG CTGGACCACC   750
TGAGTCAGCA GAATCTACAG ATGCCCTCAA GCTTTGTCCT CATGAAGAAT   800
TCCTGAGACT ATGTAAAGAA AGAGCTGAAG AGATCTACCC AATAAAGGAG   850
AGAAACAACC GCACACGCCT GGCTCTCATC ATATGCAATA CAGAGTTTGA   900
CCATCTGCCT CCGAGGAATG GAGCTGACTT GACATCACAG GATGAAGGAG   950
TACTTGAGGG TCTGGACTAT GTGTAGATGT GAAGAGAATC GACAGCCAGG  1000
ATATGGAGTC AGCGCTGAGG GCATTTGCTA CCAGACCAGA GCACAAGTCC  1050
TCTGACAGCA CATTCTTGGT ACTCATGTCT CATGGCATCC TGGAGGGAAT  1100
CTGCGGAACT GTGCATGATG AGAAAAAACC AGATGTGCTG CTTTATGACA  1150
CCATCTTCCA GATATTCAAC AACCGCAACT GCCTCAGTCT GAAGGACAAA  1200
CCCAAGGTCA TCATTGTCCA GGCCTGCAGA GGTGCAAACC GTGGGGAACT  1250
GTGGGTCAGA GACTCTCCAG CATCCTTGGA AGTGGCCTCT TCACAGTCAT  1300
CTGAGAACCT GGAGGAAGAT GCTGTTTACA AGACCCACGT GGAGAAGGAC  1350
TTCATTGCTT TCTGCTCTTC AACGCCACAC AACGTGTCCT GGAGAGACAG  1400
CACAATGGGC TCTATCTTCA TCACACAACT CATCACATGC TTCCAGAAAT  1450
ATTCTTGGTG CTGCCACCTA GAGGAAGTAT TTCGGAAGGT ACAGCAATCA  1500
TTTGAAACTC CAAGGGCCAA AGCTCAAATG CCCACCATAG AACGACTGTC  1550
CATGACAAGA TATTTCTACC TCTTTCCTGG CAATTGA                1587
```

(10) INFORMATION FOR SEQ ID NO: 9:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 1894
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE:
(A) DESCRIPTION: DNA

(ix) FEATURE:
(A) NAME/KEY: chimeric gene TNFp1005 AIG.2
(D) OTHER INFORMATION: residues 1 to 1024 comprise the promoter sequence; residues 1025 to 1036, the linker sequence, and the remaining residues comprise the AIG.2 sequence

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 9:

```
GGCGGGGGTC AGGGAGCTCC TGGGAGATAT GGCCACATGT AGCGGCTCTG     50
AGGAATGGGT TACAGGAGAC CTCTGGGGAG ATGTGACCAC AGCAATGGGT    100
AGGAGAATGT CCAGGGCTAT GGAAGTCGAG TATGGGGACC CCCCCTTAAC    150
GAAGACAGGG CCATGTAGAG GGCCCCAGGG AGTGAAAGAG CCTCCAGGAC    200
CTCCAGGTAT GGAATACAGG GGACGTTTAA GAAGATATGG CCACACACTG    250
GGGCCCTGAG AAGTGAGAGC TTCATGAAAA AAATCAGGGA CCCCAGAGTT    300
CCTTGGAAGC CAAGACTGAA ACCAGCATTA TGAGTCTCCG GGTCAGAATG    350
AAAGAAGAAG GCCTGCCCCA GTGGGGTCTG TGAATTCCCG GGGGTGATTT    400
```

CACTCCCCGG GGCTGTCCCA GGCTTGTCCC TGCTACCCCC ACCCAGCCTT 450
TCCTGAGGCC TCAAGCCTGC CACCAAGCCC CCAGCTCCTT CTCCCCGCAG 500
GGACCCAAAC ACAGGCCTCA GGACTCAACA CAGCTTTTCC CTCCAACCCC 550
GTTTTCTCTC CCTCAAGGAC TCAGCTTTCT GAAGCCCCTC CCAGTTCTAG 600
TTCTATCTTT TTCCTGCATC CTGTCTGGAA GTTAGAAGGA AACAGACCAC 650
AGACCTGGTC CCCAAAAGAA ATGGAGGCAA TAGGTTTTGA GGGGCATGGG 700
GACGGGGTTC AGCCTCCAGG GTCCTACACA CAAATCAGTC AGTGGCCCAG 750
AAGACCCCCC TCGGAATCGG AGCAGGGAGG ATGGGGAGTG TGAGGGGTAT 800
CCTTGATGCT TGTGTGTCCC CAACTTTCCA AATCCCCGCC CCCGCGATGG 850
AGAAGAAACC GAGACAGAAG GTGCAGGGCC CACTACCGCT TCCTCCAGAT 900
GAGCTCATGG GTTTCTCCAC CAAGGAAGTT TTCCGCTGGT TGAATGATTC 950
TTTCCCCGCC CTCCTCTCGC CCCAGGGACA TATAAAGGCA GTTGTTGGCA 1000
CACCCAGCCA GCAGACGCTC CCTCAGCAGA TCCACCATGG CTGGACCACC 1050
TGAGTCAGCA GAATCTACAG ATGCCCTCAA GCTTTGTCCT CATGAAGAAT 1100
TCCTGAGACT ATGTAAAGAA AGAGCTGAAG AGATCTACCC AATAAAGGAG 1150
AGAAACAACC GCACACGCCT GGCTCTCATC ATATGCAATA CAGAGTTTGA 1200
CCATCTGCCT CCGAGGAATG GAGCTGACTT TGACATCACA GGGATGAAGG 1250
AGCTACTTGA GGGTCTGGAC TATAGTGTAG ATGTAGAAGA GAATCTGACA 1300
GCCAGGGATA TGGAGTCAGC GCTGAGGGCA TTTGCTACCA GACCAGAGCA 1350
CAAGTCCTCT GACAGCACAT TCTTGGTACT CATGTCTCAT GGCATCCTGG 1400
AGGGAATCTG CGGAACTGTG CATGATGAGA AAAAACCAGA TGTGCTGCTT 1450
TATGACACCA TCTTCCAGAT ATTCAACAAC CGCAACTGCC TCAGTCTGAA 1500
GGACAAACCC AAGGTCATCA TTGTCCAGGC CTGCAGAGGT GCAAACCGTG 1550
GGGAACTGTG GGTCAGAGAC TCTCCAGCAT CCTTGGAAGT GGCCTCTTCA 1600
CAGTCATCTG AGAACCTGGA GGAAGATGCT GTTTACAAGA CCCACGTGGA 1650
GAAGGACTTC ATTGCTTTCT GCTCTTCAAC GCCACACAAC GTGTCCTGGA 1700
GAGACAGCAC AATGGGCTCT ATCTTCATCA CACAACTCAT CACATGCTTC 1750
CAGAAATATT CTTGGTGCTG CCACCTAGAG GAAGTATTTC GGAAGGTACA 1800
GCAATCATTT GAAACTCCAA GGGCCAAAGC TCAAATGCCC ACCATAGAAC 1850
GACTGTCCAT GACAAGATAT TTCTACCTCT TTCCTGGCAA TTGA 1894

(11) INFORMATION FOR SEQ ID NO: 10:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 123
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE:
(A) DESCRIPTION: DNA

(ix) FEATURE:
(A) NAME/KEY: TNFα promoter enhancer region 1 (ER1)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 10:

GGGGCGGGGG TCAGGGAGCT CCTGGGAGAT ATGGCCACAT GTAGCGGCTC 50
TGAGGAATGG GTTACAGGAG ACCTCTGGGG AGATGTGACC ACAGCAATGG 100
GTAGGAGAAT GTCCAGGGCT ATG 123

(12) INFORMATION FOR SEQ ID NO: 11:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 190
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE:
(A) DESCRIPTION: DNA

(ix) FEATURE:
(A) NAME/KEY: TNFα promoter enhancer region 2 (ER2)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 11:

```
TCCTTGGAAG CCAAGACTGA AACCAGCATT ATGAGTCTCC GGGTCAGAAT     50
GAAAGAAGAA GGCCTGCCCC AGTGGGGTCT GTGAATTCCC GGGGGTGATT    100
TCACTCCCCG GGGCTGTCCC AGGCTTGTCC CTGCTACCCC CACCCAGCCT    150
TTCCTGAGGC CTCAAGCCTG CCACCAAGCC CCCAGCTCCT               190
```

(13) INFORMATION FOR SEQ ID NO: 12:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 223
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE:
(A) DESCRIPTION: DNA

(ix) FEATURE:
(A) NAME/KEY: multiple conling sites
(D) OTHER INFORMATION: genetically engineered multiple clonging sites genetically engineered upstream of the minimal TNFα promoter in the -120pGL3 construct

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 12:

```
GGTACCGAGC TCTTACGCGT GCTAGCCGCG GATATCTTAA GACGTCCTAG     50
GACTAGTCAG CTGCTCGAGC CGCTTCCTCC AGATGAGCTC ATGGGTTTCT    100
CCACCAAGGA AGTTTTCCGC TGGTTGAATG ATTCTTTCCC CGCCCTCCTC    150
TCGCCCCAGG GACATATAAA GGCAGTTGTT GGCACACCCA GCCAGCAGAC    200
GCTCCCTCAG CAGATCTAAG CTT                                223
```

(14) INFORMATION FOR SEQ ID NO: 13:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 787
(B) TYPE: nucleic acid

(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE:
(A) DESCRIPTION: DNA

(ix) FEATURE:
(A) NAME/KEY: TNFα intranslated region

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 13:

```
TCTAGAGGAG GACGAACATC CAACCTTCCC AAACGCCTCC CCTGCCCCAA     50
TCCCTTTATT ACCCCCTCCT TCAGACACCC TCAACCTCTT CTGGCTCAAA    100
AAGAGAATTG GGGGCTTAGG GTCGGAACCC AAGCTTAGAA CTTTAAGCAA    150
CAAGACCACC ACTTCGAAAC CTGGGATTCA GGAATGTGTG GCCTGCACAG    200
TGAAGTGCTG GCAACCACTA AGAATTCAAA CTGGGGCCTC CAGAACTCAC    250
TGGGGCCTAC AGCTTTGATC CCTGACATCT GGAATCTGGA GACCAGGGAG    300
CCTTTGGTTC TGGCCAGAAT GCTGCAGGAC TTGAGAAGAC CTCACCTAGA    350
AATTGACACA AGTGGACCTT AGGCCTTCCT CTCTCCAGAT GTTTCCAGAC    400
TTCCTTGAGA CACGGAGCCC AGCCCTCCCC ATGGAGCCAG CTCCCTCTAT    450
TTATGTTTGC ACTTGTGATT ATTTATTATT TATTTATTAT TTATTTATTT    500
ACAGATGAAT GTATTTATTT GGGAGACCGG GGTATCCTGG GGGACCCAAT    550
GTAGGAGCTG CCTTGGCTCA GACATGTTTT CCGTGAAAAC GGAGCTGAAC    600
AATAGGCTGT TCCCATGTAG CCCCCTGGCC TCTGTGCCTT CTTTTGATTA    650
TGTTTTTTAA AATATTTATC TGATTAAGTT GTCTAAACAA TGCTGATTTG    700
GTGACCAACT GTCACTCATT GCTGAGCCTC TGCTCCCCAG GGGAGTTGTG    750
TCTGTAATCG CCCTACTATT CAGTGGCGAG ATCTAGA                  787
```

CLAIMS

1. A chimeric gene comprising at least one TNFα promoter enhancer attached to a functional copy of a minimal TNFα promoter and further attached to at least one copy of an apoptosis-inducing gene, wherein the expression of the apoptosis-inducing gene is driven by the TNFα promoter.

2. A gene according to claim 1 wherein the attachment of the enhancer to the promoter and the promoter to the apoptosis-inducing gene is selected from the group consisting of direct attachment, distal attachment, proximal attachment, and combinations thereof.

3. A gene according to claim 1 comprising 2 or more copies of the TNFα promoter enhancer.

4. A gene according to claim 1 wherein the TNFα promoter enhancer is SEQ ID NO: 10 or SEQ ID NO: 11, or functional fragments or variants thereof.

5. A gene according to claim 1 wherein the TNFα promoter is selected from the group consisting of SEQ ID NO: 1, SEQ ID NO: 2, SEQ ID NO: 3, and functional fragments or variants thereof.

6. A gene according to claim 1 wherein the apoptosis-inducing gene is selected from the group consisting of caspase-1, caspase-2, caspase-3, caspase-4, caspase-5, caspase-6, caspase-7, caspase-8, caspase-9, caspase-10, Granzyme A, Granzyme B, Fas ligand, and functional fragments, variants, and mixtures of any of these.

7. A gene according to claim 6 wherein the apoptosis-inducing gene is selected from the group consisting of caspase 3, caspase 4, caspase 5, Granzyme B, and functional fragments, variants, and mixtures of any of these.

8. A gene according to claim 1 selected from the group consisting of SEQ ID NO: 4, SEQ ID NO: 5, SEQ ID NO: 6, SEQ ID NO: 7, SEQ ID NO: 8, SEQ ID NO: 9, and functional fragments or variants thereof.

9. A gene according to claim 1 selected from the group consisting of SEQ ID NO: 4, SEQ ID NO: 5, SEQ ID NO: 6, SEQ ID NO: 7, SEQ ID NO: 8, SEQ ID NO: 9, and functional fragments or variants thereof, wherein the 3'UTR of the TNFα gene is ligated downstream of the apotosis-inducing gene.

10. A pharmaceutical composition comprising a gene according to claim 1.

11. A pharmaceutical composition comprising a gene according to claim 9.

12. A method for treating an inflammatory disorder in a patient comprising the step of inducing apoptosis in inflammatory cells or cells at a site of inflammation of the patient by introducing into the cells a chimeric gene according to claim 1.

13. A method according to claim 12 wherein the induction of apoptosis does not induce an inflammatory response in the patient.

14. A method according to claim 12 wherein the inflammatory cell is a TNFα producing cell.

15. A method according to claim 12 wherein the inflammatory disorder is selected from the group consisting of rheumatoid arthritis, multiple sclerosis, Guillain-Barre syndrome, Crohn's disease, ulcerative colitis, psoriasis, graft versus

host disease, lupus erythematosus, insulin-dependent diabetes mellitus, psoriatic arthritis, sarcoidosis, hypersensitivity pneumonitis, ankylosing spondylitis, Reiter's syndrome, and systemic sclerosis.

16. A method according to claim 15 wherein the inflammatory disorder is rheumatoid arthritis.

17. A chimeric gene comprising 2 to 10 cassettes of a TNFα promoter enhancer attached to at least one copy of a minimal TNFα promoter, and at least one copy of an apoptosis-inducing gene selected from the group consisting of caspase 3, caspase 4, caspase 5, Granzyme B and functional fragments, variants, and mixtures of any of these, wherein the expression of the apoptosis-inducing gene is driven by the TNF promoter.

18. A pharmaceutical composition comprising a gene according to claim 17.

19. A method for inducing apoptosis in a TNFα-producing inflammatory cell by introducing into the cell a chimeric gene according to claim 17.

20. A method for treating an inflammatory disorder in a patient comprising inducing apoptosis in inflammatory cells or cells at the site of inflammation of the patient by introducing into the cells a chimeric gene according to claim 17 without inducing an inflammatory response in the patient.

21. A process for constructing a chimeric gene comprising at least one TNFα promoter enhancer attached to a functional copy of a minimal TNFα promoter and further attached to at least one copy of an apoptosis-inducing gene, wherein the expression of the apoptosis-inducing gene is driven by the TNFα promoter comprising the steps of

(a) amplifying a TNFα promoter by a polymerase chain reaction using primers encompassing deletion constructs of the TNFα promoter;

(b) cloning the PCR-amplified genes obtained in step (a) upstream of a reporter gene;

(c) testing the constructs obtained in step (b) for their constitutive and inducible expression in at least one TNFα-producing cell line;

(d) selecting TNFα promoter responsible for inducible expression of the reporter in the cell line; and either

(e) PCR-amplifying TNFα promoter regions that enhance expression of the reporter to obtain an enhancer and ligating at least one copy of the enhancer upstream of the promoter; or

(f) inserting at least one copy of a prodomain-deleted apoptosis-inducing gene downstream of the TNFα promoter by replacing the reporter gene with the apoptosis-inducing gene deletion constructs to obtain a chimeric gene or

(g) PCR-amplifying a TNFα-3'UTR and ligating downstream of the reporter gene, or any combination of these procedures.

22. A process according to claim 21 wherein 2 or more copies of the enhancer are inserted upstream of the promoter.

23. A process according to claim 21 wherein reporter gene is luciferase.

24. A process according to claim 21 wherein the enhancer comprises SEQ ID NO: 10 or SEQ ID NO: 11.

25. A process according to claim 21 wherein the prodomain-deleted apoptosis-inducing gene is selected from the group consisting of caspase 3, caspase 4, caspase 5, Granzyme B, and functional fragments and variants thereof.

27. A process according to claim 21 producing a gene selected from the group consisting of SEQ ID NO: 4, SEQ ID NO: 5, SEQ ID NO: 6, SEQ ID NO: 7, SEQ ID NO: 8, SEQ ID NO: 9, and functional fragments or variants thereof.

28. A process according to claim 21 wherein the cell lines for testing constructs are selected from the group consisting of T lymphoblastoid, myelomonocytic, monocytic, fibroblast, and cultured human synoviocytes.

29. A chimeric gene comprising:

(a) at least one promoter enhancer attached to a functional copy of a minimal promoter, provided that the promoter is a gene or combination of genes activated in inflammatory cells or in cells at a site of inflammation, and

(b) further attached to at least one copy of an apoptosis-inducing gene, wherein the expression of the apoptosis-inducing gene is driven by the promoter, and the promoter is selected from the group consising of cytokines, interleukins and their receptors, cell adhesion molecules and their ligands, chemokines and their receptors, pro-inflammatory enzymes, and mixtures thereof.

31. A gene according to claim 29 wherein the promoter is selected from the group consisting of TNFβ, IL-1α, IL-1β, IL-2, IL-6, IL-8, GM-CSF, interferonγ, functional fragments and variants thereof, and mixtures of any of these.

32. A gene according to claim 29 wherein the promoter is selected from the group consisting of selectins, integrins, ICAM-1, V-CAM, functional fragments and variants thereof, and mixtures of any of these.

33. A gene according to claim 29 wherein the promoter is selected from the group consisting of MIP-1α, MIP-1β; MCP1-4, RANTES, Mig, NAP2, IP10, Gro α-γ, functional fragments and variants thereof, and mixtures of any of these.

34. A gene according to claim 29 wherein the promoter is selected from the group consisting of COX-2, iNOS, phospholipases, proteases, functional fragments and variants thereof, and mixtures of any of these.

35. A gene according to claim 29 wherein the attachment of the enhancer to the promoter and the promoter to the apoptosis-inducing gene is selected from the group consisting of direct attachment, distal attachment, proximal attachment, and combinations thereof.

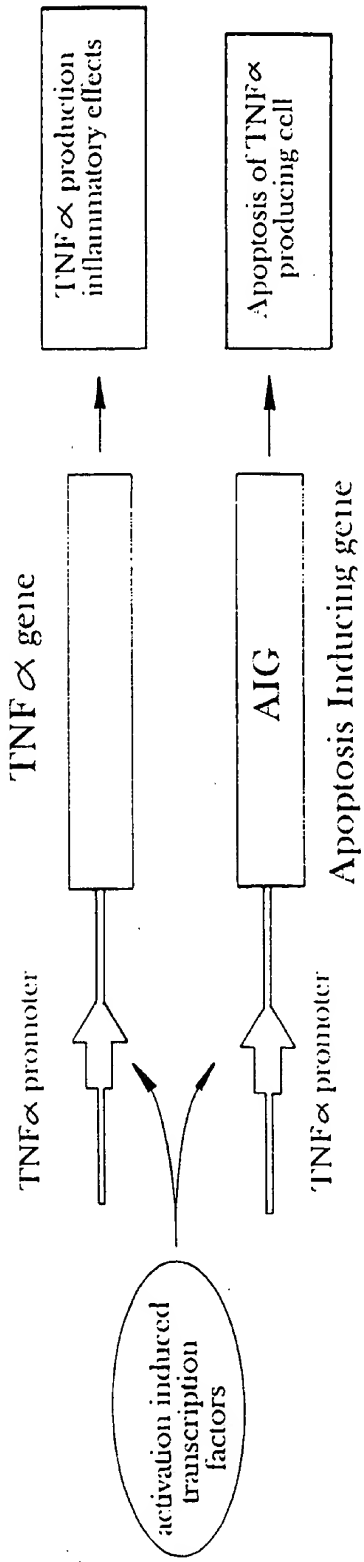
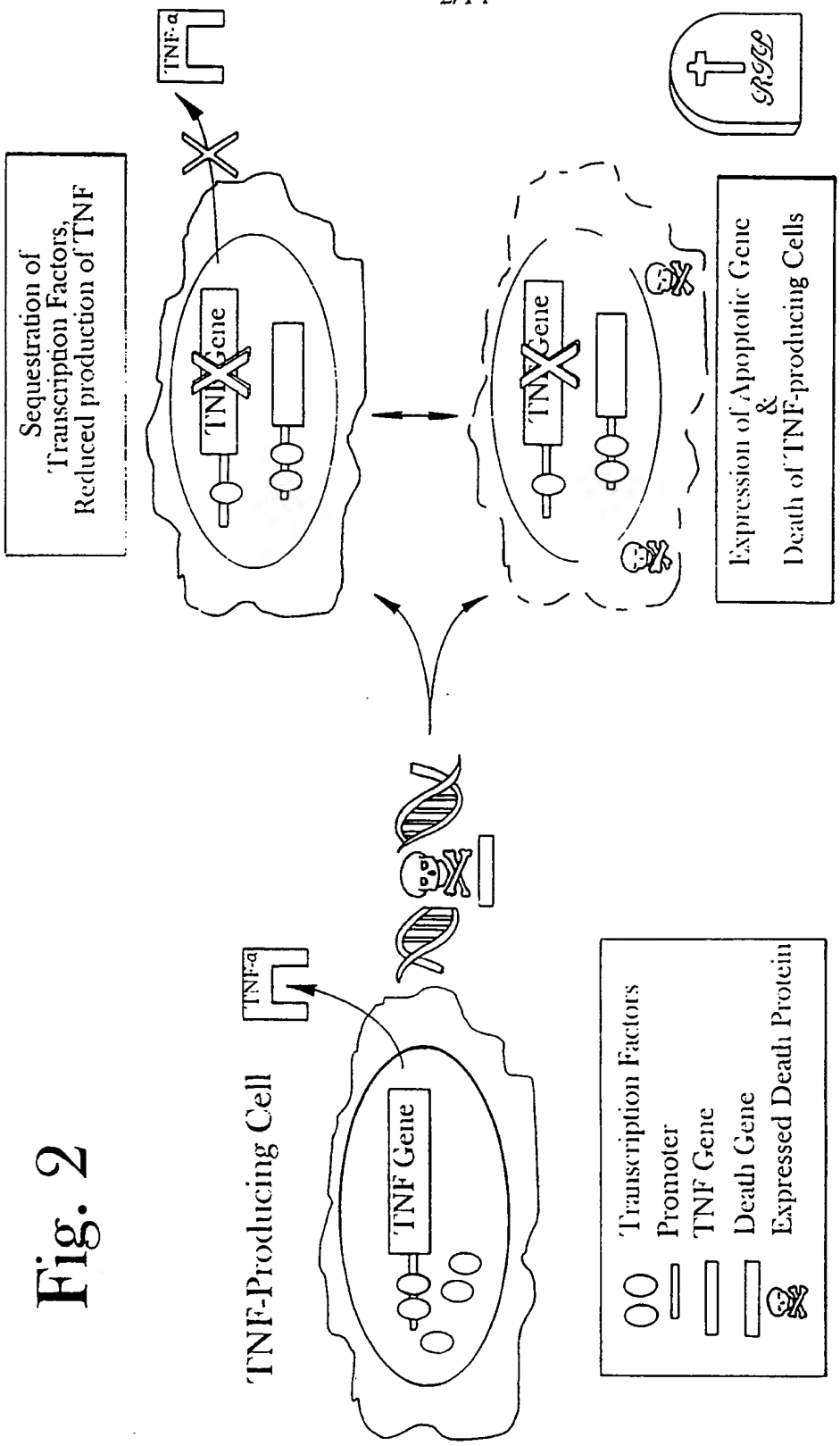

TNFα production
inflammatory effects
Apoptosis of TNFα
producing cell
TNFα gene
AIG
Apoptosis Inducing gene
Caspase
(1-10)
Granzyme B
FasL
TNFα promoter
TNFα promoter
activation induced
transcription
factors

Fig. 1

Fig. 2


TNF-Producing Cell
TNF-α
TNF Gene
Transcription Factors
Promoter
TNF Gene
Death Gene
Expressed Death Protein
TNF Gene
TNF Gene
TNF-α
Sequestration of Transcription Factors, Reduced production of TNF
Expression of Apoptotic Gene & Death of TNF-producing Cells

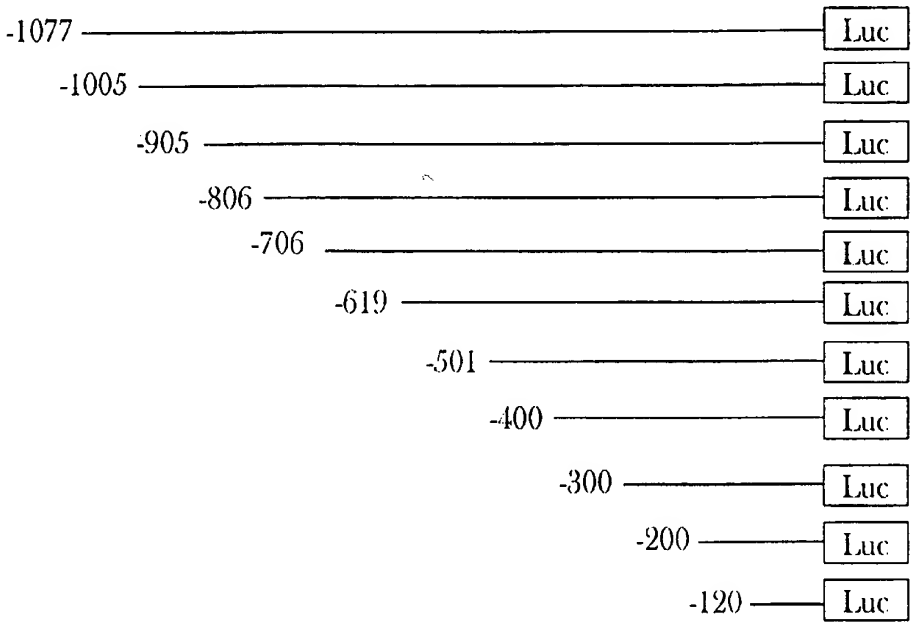
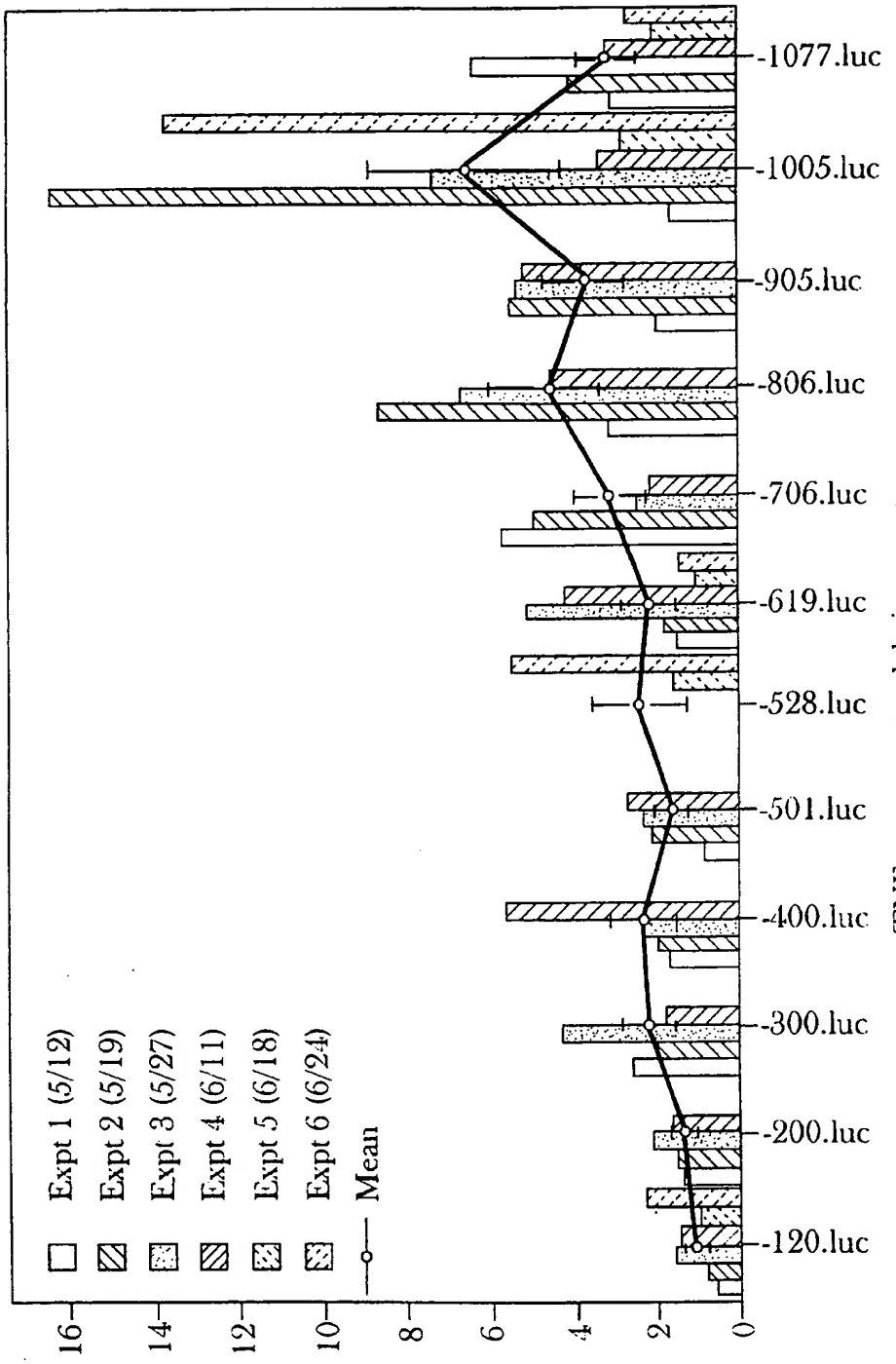

-1077
Luc
-1005
Luc
-905
Luc
-806
Luc
-706
Luc
-619
Luc
-501
Luc
-400
Luc
-300
Luc
-200
Luc
-120
Luc

Fig. 3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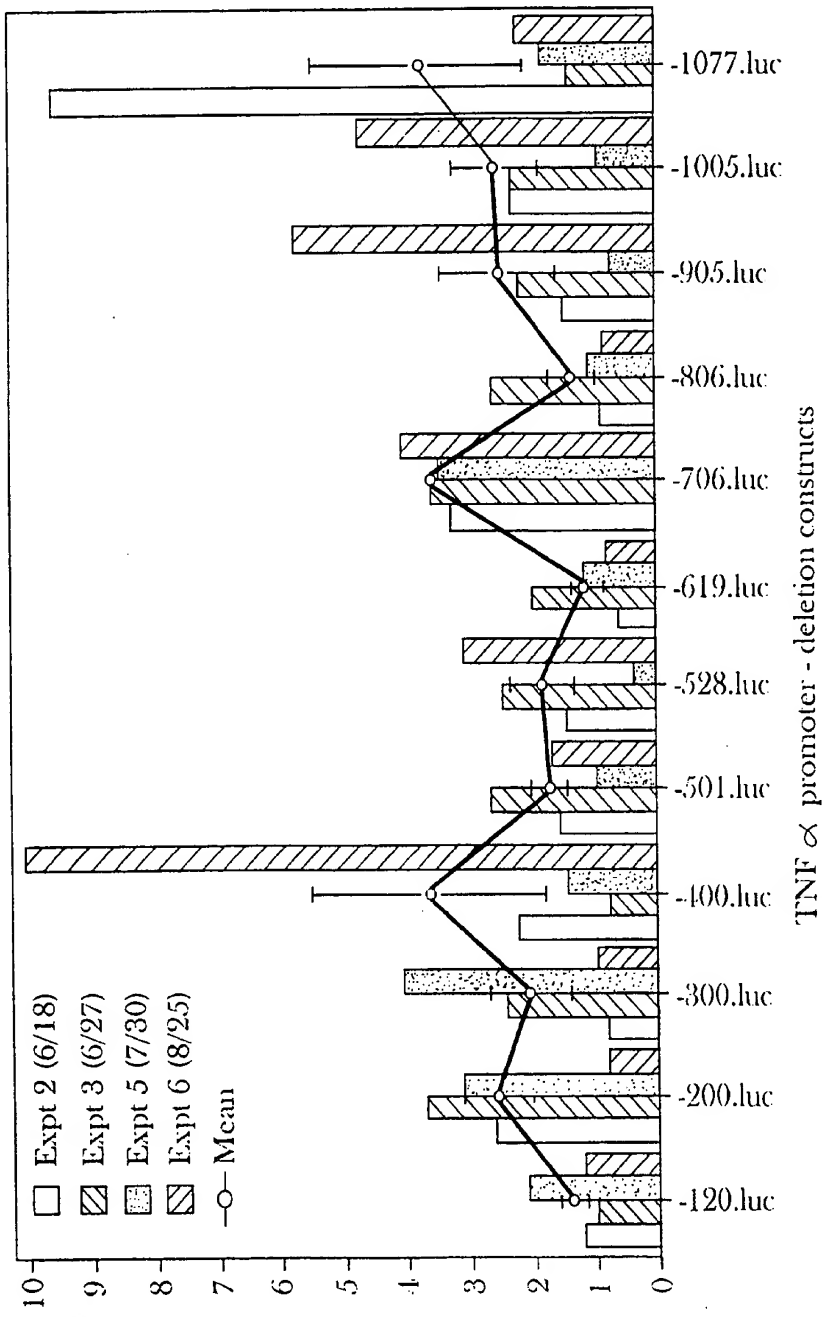
-1005.luc
-905.luc
-806.luc
-706.luc
-619.luc
-528.luc
-501.luc
-400.luc
-300.luc
-200.luc
-120.luc
TNFα promoter - deletion constructs
Expt 1 (5/12)
Expt 2 (5/19)
Expt 3 (5/27)
Expt 4 (6/11)
Expt 5 (6/18)
Expt 6 (6/24)
Mean
16
14
12
10
8
6
4
2
0
PMA - inducible increase in Luciferase activity

Fig. 4a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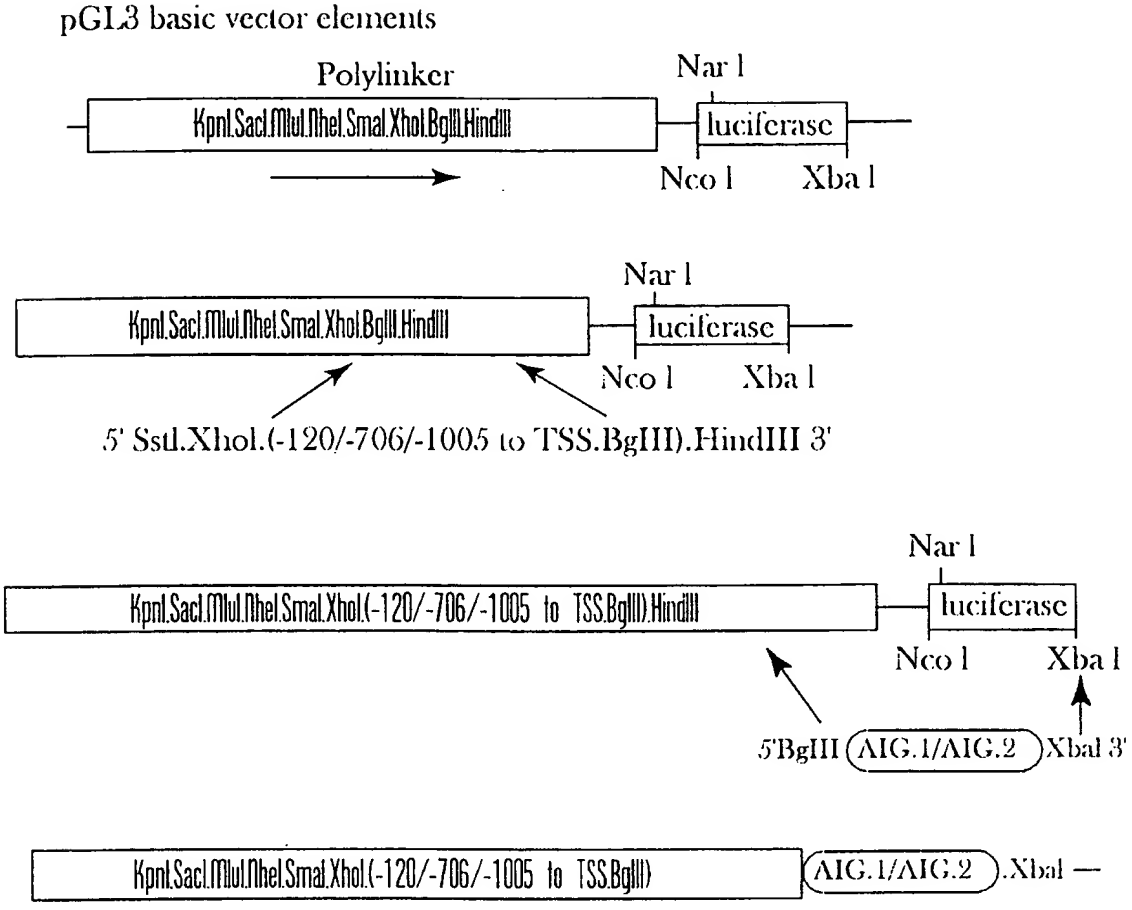
-1005.luc
-905.luc
-806.luc
-706.luc
-619.luc
-528.luc
-501.luc
-400.luc
-300.luc
-200.luc
-120.luc
TNF ∝ promoter - deletion constructs
Expt 2 (6/18)
Expt 3 (6/27)
Expt 5 (7/30)
Expt 6 (8/2.5)
Mean
10
9
8
7
6
5
4
3
2
1
0
LPS - inducible increase in Luciferase activity

Fig. 4b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Nar I
KpnI.SacI.MluI.NheI.SmaI.XhoI.BglII.HindIII
luciferase
Nco I
Xba I
Nar I
KpnI.SacI.MluI.NheI.SmaI.XhoI.BglII.HindIII
luciferase
Nco I
Xba I
5' SstI.XhoI.(-120/-706/-1005 to TSS.BglII).HindIII 3'
Nar I
KpnI.SacI.MluI.NheI.SmaI.XhoI.(-120/-706/-1005 to TSS.BglII).HindIII
luciferase
Nco I
Xba I
5'BglII
AIG.1/AIG.2
XbaI 3'
KpnI.SacI.MluI.NheI.SmaI.XhoI.(-120/-706/-1005 to TSS.BglII)
AIG.1/AIG.2
.XbaI

Fig. 5

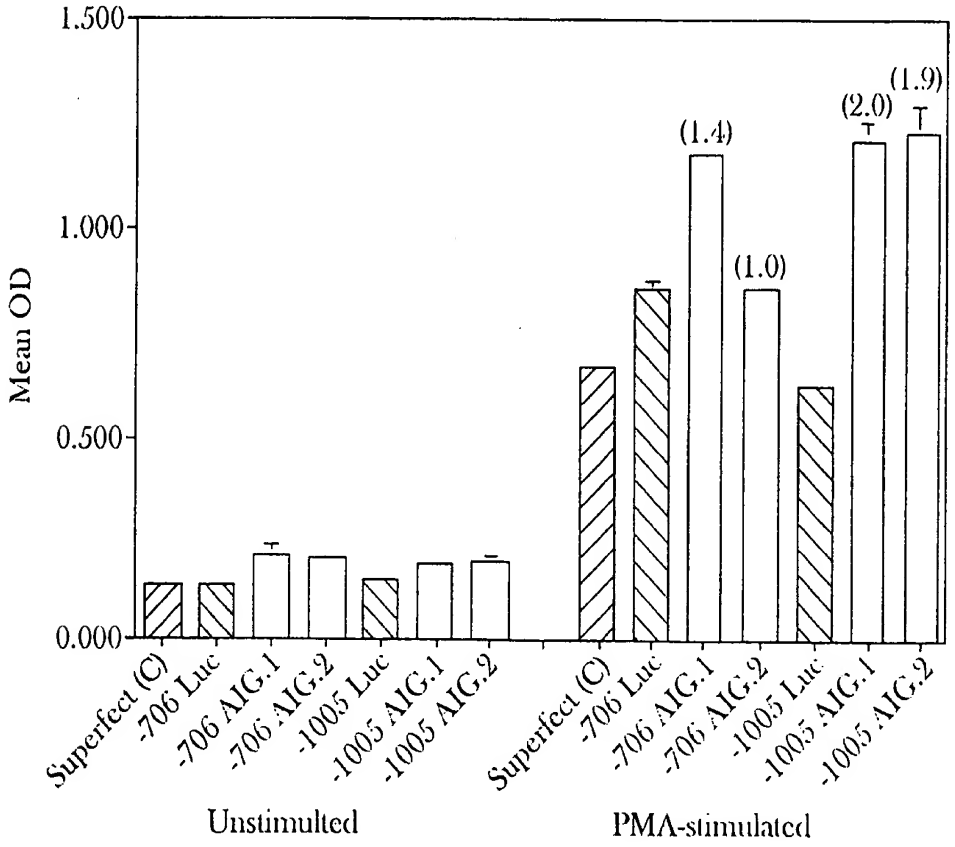

1.500
1.000
0.500
0.000
Mean OD
(1.4)
(1.0)
(2.0)
(1.9)
Superfect (C)
-706 Luc
-706 AIG.1
-706 AIG.2
-1005 Luc
-1005 AIG.1
-1005 AIG.2
Superfect (C)
-706 Luc
-706 AIG.1
-706 AIG.2
-1005 Luc
-1005 AIG.1
-1005 AIG.2
Unstimulted
PMA-stimulated

Fig. 6a

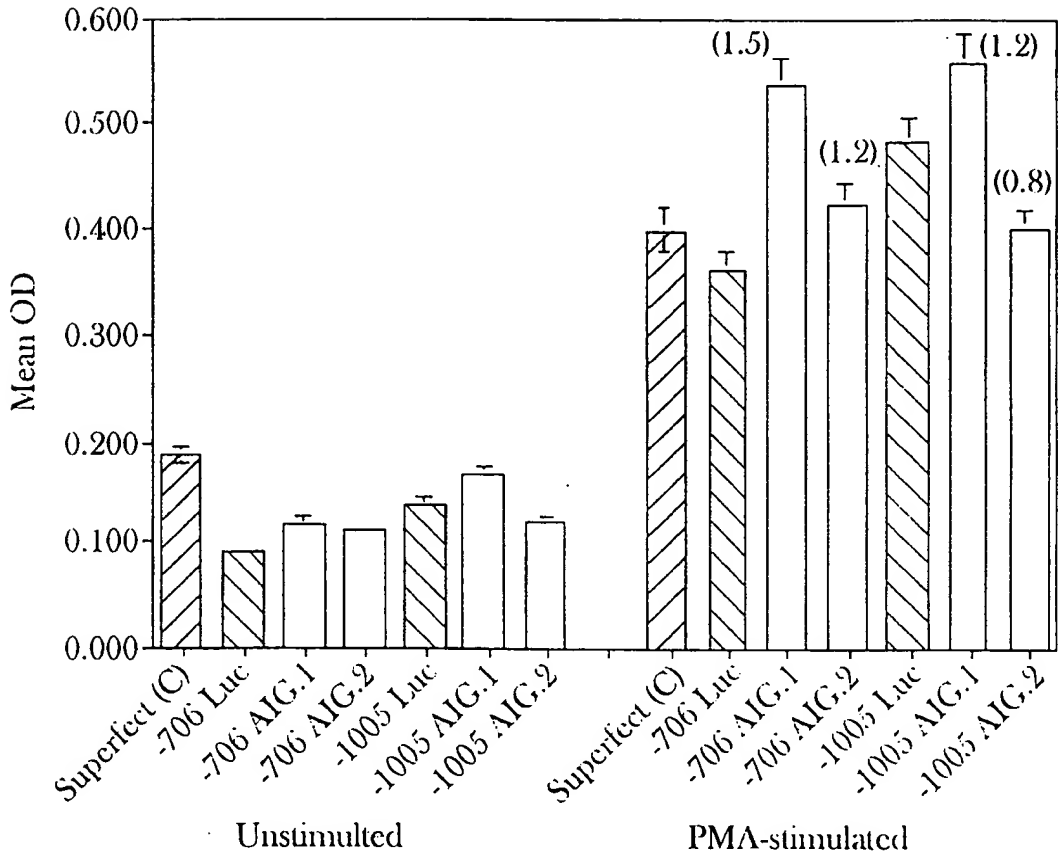

0.600
0.500
0.400
0.300
0.200
0.100
0.000
Mean OD
(1.5)
(1.2)
(1.2)
(0.8)
Superfect (C)
-706 Luc
-706 AIG.1
-706 AIG.2
-1005 Luc
-1005 AIG.1
-1005 AIG.2
Superfect (C)
-706 Luc
-706 AIG.1
-706 AIG.2
-1005 Luc
-1005 AIG.1
-1005 AIG.2
Unstimulted
PMA-stimulated

Fig. 6b

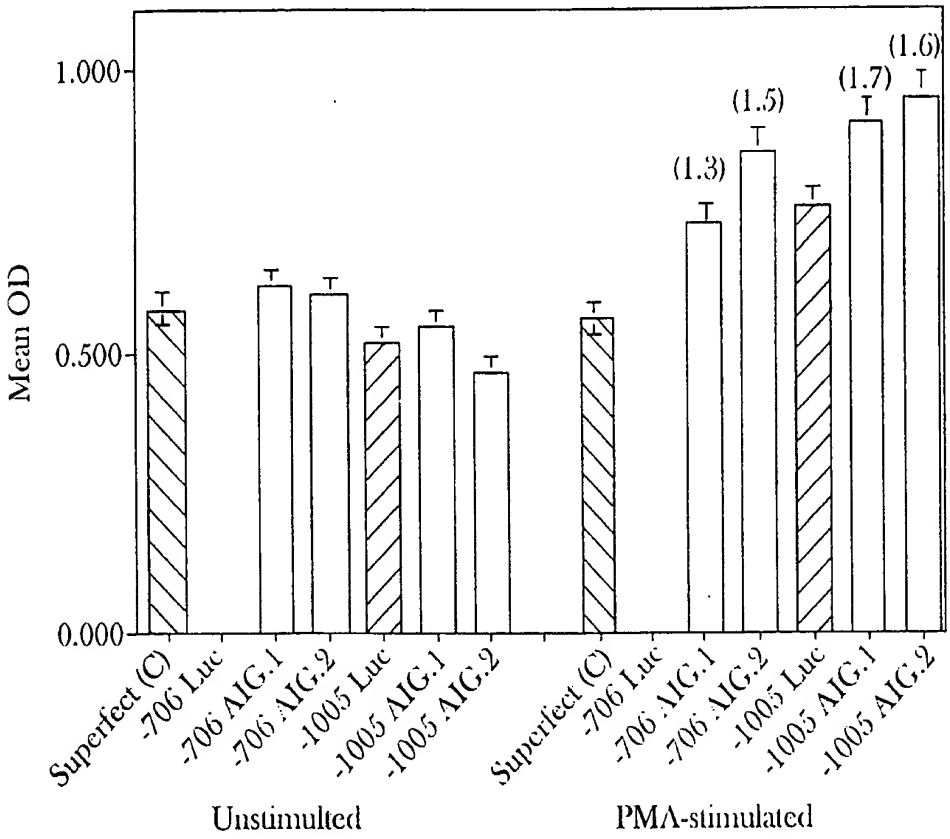

1.000
0.500
0.000
Mean OD
(1.3)
(1.5)
(1.7)
(1.6)
Superfect (C)
-706 Luc
-706 AIG.1
-706 AIG.2
-1005 Luc
-1005 AIG.1
-1005 AIG.2
Superfect (C)
-706 Luc
-706 AIG.1
-706 AIG.2
-1005 Luc
-1005 AIG.1
-1005 AIG.2
Unstimulted
PMA-stimulated

Fig. 6c

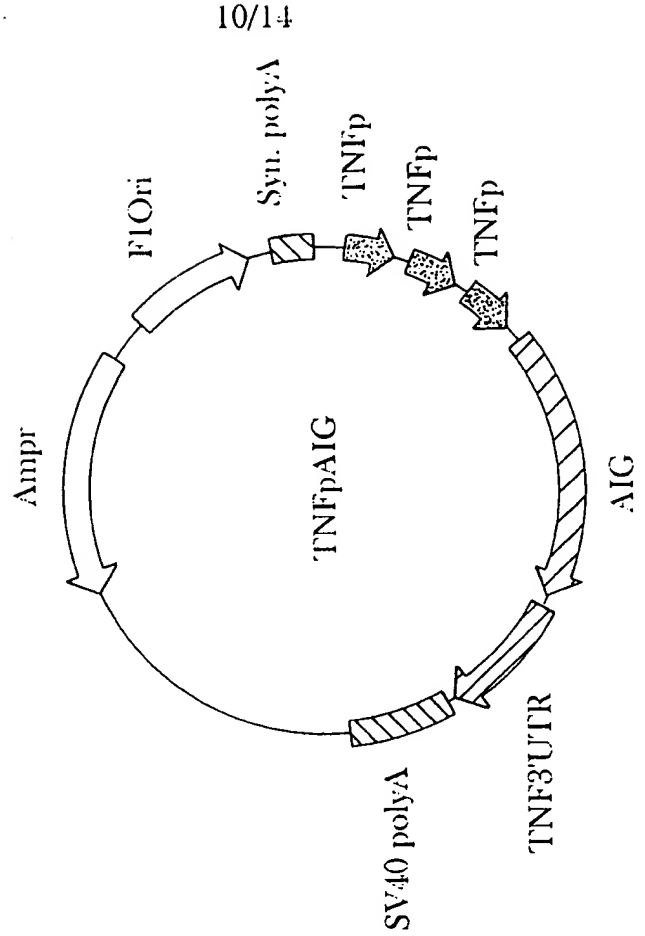

Ampr
F1Ori
Syn. polyA
TNFp
TNFp
TNFp
AIG
TNF3'UTR
SV40 polyA
TNFpAIG

Fig. 7B

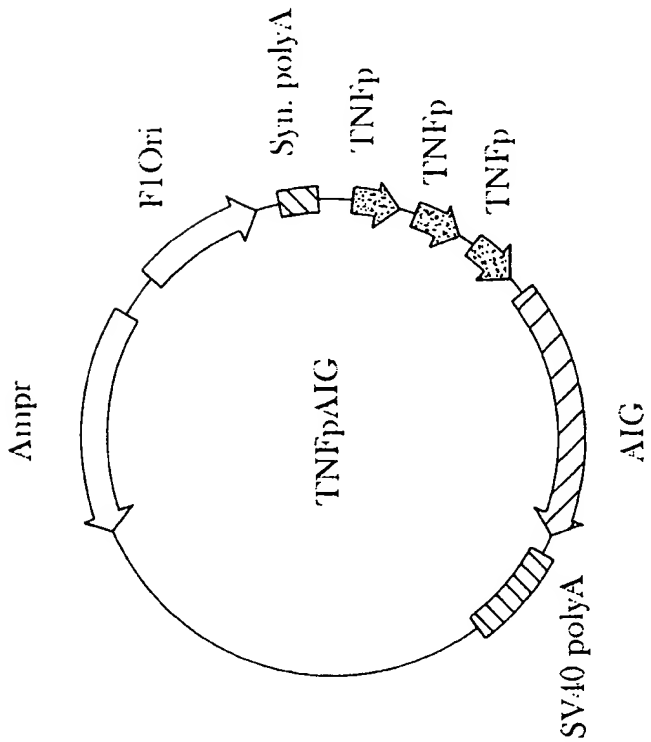
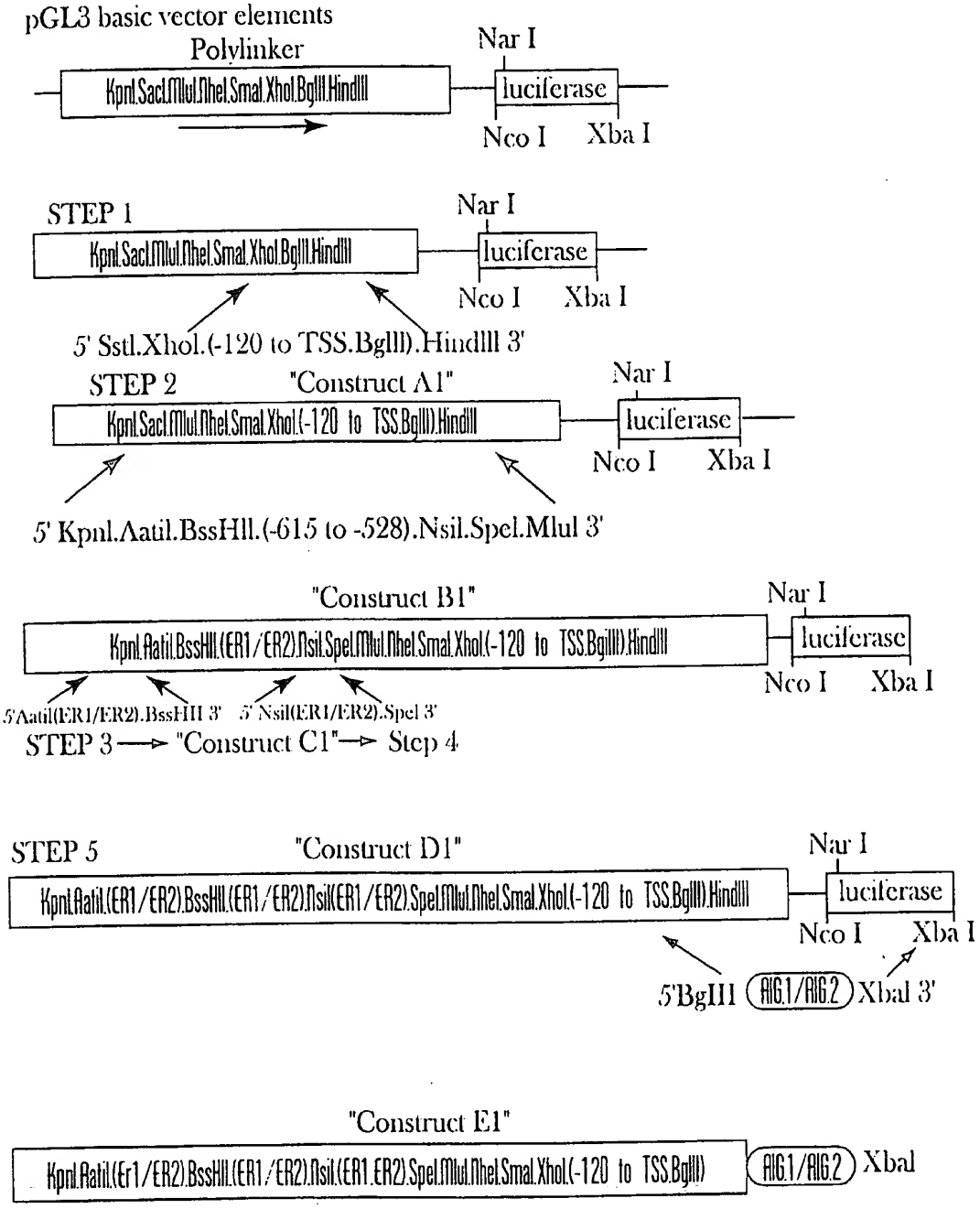

Ampr
F1Ori
Syn. polyA
TNFp
TNFp
TNFp
AIG
SV40 polyA
TNFpAIG

Fig. 7A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Polylinker
KpnI.SacI.MluI.NheI.SmaI.XhoI.BglII.HindIII
Nar I
luciferase
Nco I
Xba I
STEP 1
5' SstI.XhoI.(-120 to TSS.BglII).HindIII 3'
STEP 2
"Construct A1"
5' KpnI.AatII.BssHII.(-615 to -528).NsiI.SpeI.MluI 3'
"Construct B1"
5'AatII(ER1/ER2).BssHII 3'
5' NsiI(ER1/ER2).SpeI 3'
STEP 3 → "Construct C1" → Step 4
STEP 5
"Construct D1"
5'BglII
AIG1/AIG2
XbaI 3'
"Construct E1"
XbaI

Fig. 8a

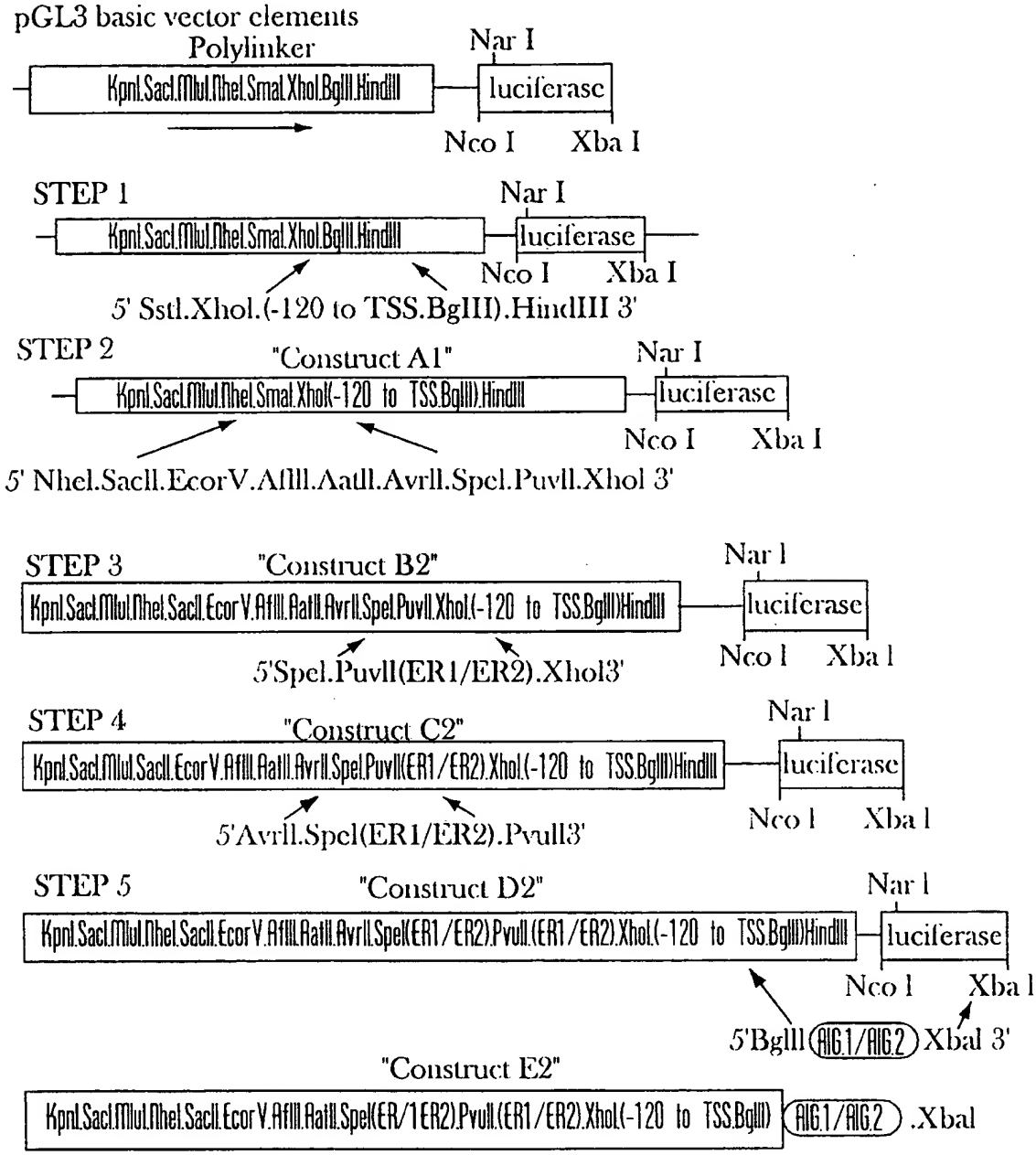
pGL3 basic vector elements
Polylinker
Nar I
KpnI.SacI.MluI.NheI.SmaI.XhoI.BglII.HindIII
luciferase
Nco I
Xba I
STEP 1
Nar I
KpnI.SacI.MluI.NheI.SmaI.XhoI.BglII.HindIII
luciferase
Nco I
Xba I
5' SstI.XhoI.(-120 to TSS.BglII).HindIII 3'
STEP 2
"Construct A1"
Nar I
KpnI.SacI.MluI.NheI.SmaI.XhoI(-120 to TSS.BglII).HindIII
luciferase
Nco I
Xba I
5' NheI.SacII.EcorV.AflII.AatII.AvrII.SpeI.PuvII.XhoI 3'
STEP 3
"Construct B2"
Nar I
KpnI.SacI.MluI.NheI.SacII.EcorV.AflII.AatII.AvrII.SpeI.PuvII.XhoI.(-120 to TSS.BglII)HindIII
luciferase
5'SpeI.PuvII(ER1/ER2).XhoI3'
Nco I
Xba I
STEP 4
"Construct C2"
Nar I
KpnI.SacI.MluI.SacII.EcorV.AflII.AatII.AvrII.SpeI.PuvII(ER1/ER2).XhoI.(-120 to TSS.BglII)HindIII
luciferase
5'AvrII.SpeI(ER1/ER2).PvuII3'
Nco I
Xba I
STEP 5
"Construct D2"
Nar I
KpnI.SacI.MluI.NheI.SacII.EcorV.AflII.AatII.AvrII.SpeI(ER1/ER2).PvuII.(ER1/ER2).XhoI.(-120 to TSS.BglII)HindIII
luciferase
Nco I
Xba I
5'BglII AIG1/AIG2 XbaI 3'
"Construct E2"
KpnI.SacI.MluI.NheI.SacII.EcorV.AflII.AatII.SpeI(ER/1ER2).PvuII.(ER1/ER2).XhoI.(-120 to TSS.BglII)
AIG1/AIG2
.XbaI

Fig. 8b

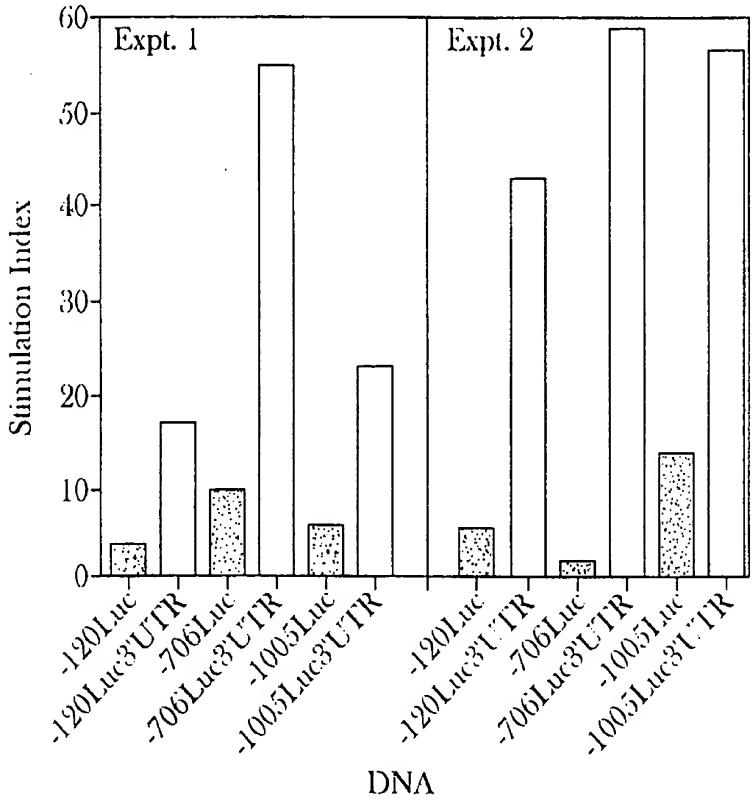
Expt. 1
Expt. 2
Stimulation Index
60
50
40
30
20
10
0
-120Luc
-120Luc3'UTR
-706Luc
-706Luc3'UTR
-1005Luc
-1005Luc3'UTR
DNA

Fig. 9

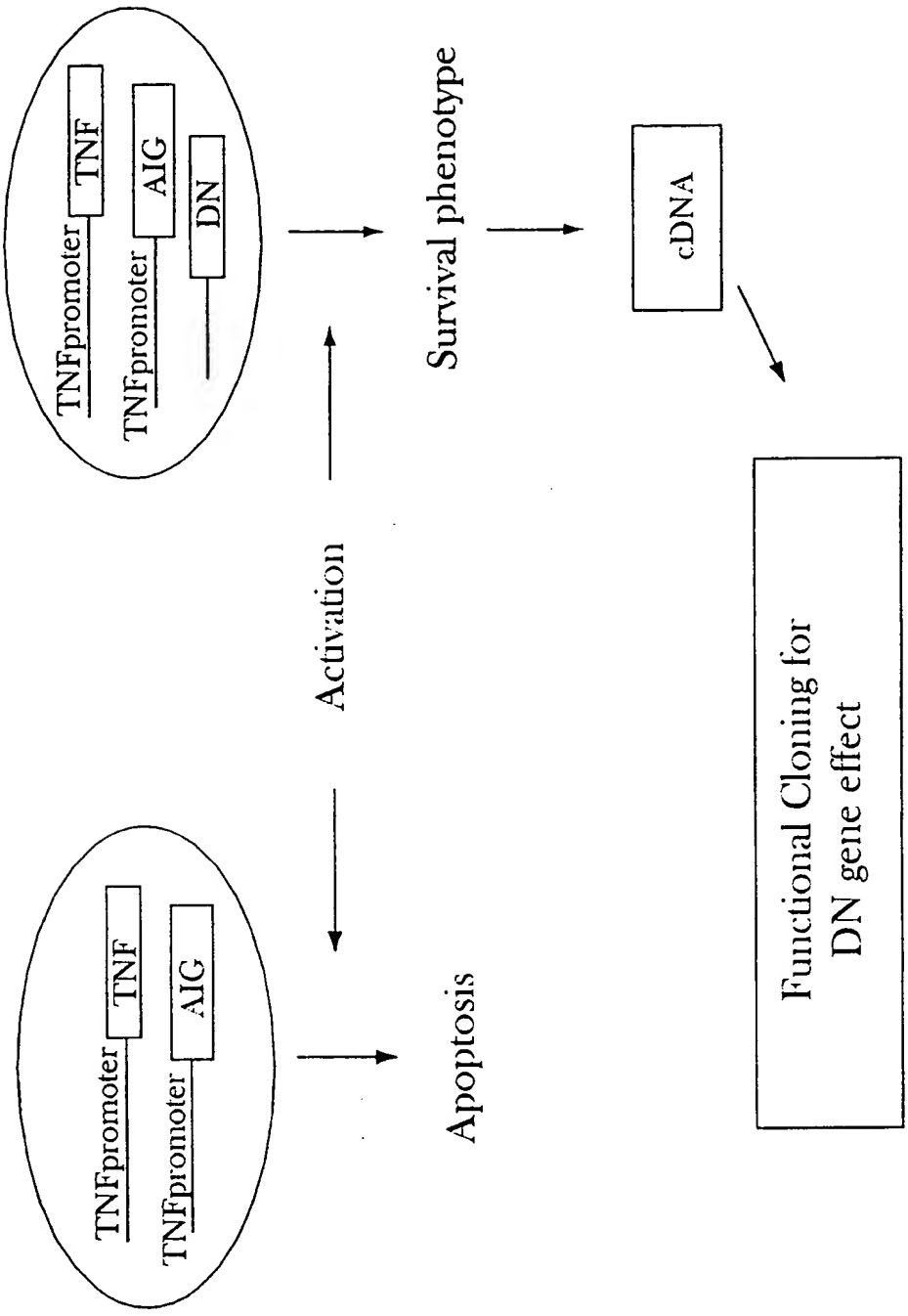
TNFpromoter
TNF
TNFpromoter
AIG
DN
Activation
Survival phenotype
cDNA
Functional Cloning for
DN gene effect
TNFpromoter
TNF
TNFpromoter
AIG
Apoptosis

Fig. 10

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US98/03911

A. CLASSIFICATION OF SUBJECT MATTER

IPC(6) :A61K 31/70; C07H 21/04; C12N 15/12; C12P 19/34
US CL :435/91.2; 514/44; 536/23.5, 24.1

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

U.S. : 435/69.1, 91.2; 514/44; 536/23.1, 23.5, 24.1

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

Please See Extra Sheet.

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	RHOADES et al. The regulation of the human tumor necrosis factor alpha promoter region in macrophage, T cell, and B cell lines. J. Biol. Chem. 05 November 1992, Vol. 267, No. 31, pages 22102-22107, see entire document.	1-35
Y,E	US 5,744,304 A (MUNFORD) 28 April 1998, columns 3-6.	1-35
A	ANDERSON, G.P. Resolution of chronic inflammation by therapeutic induction of apoptosis. Trends Pharmacol. Sci. December 1996, Vol. 17, No. 12, pages 438-442, see entire document.	1-35

[X] Further documents are listed in the continuation of Box C. [ ] See patent family annex.

*	Special categories of cited documents:	"T"	later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"A"	document defining the general state of the art which is not considered to be of particular relevance	"X"	document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"E"	earlier document published on or after the international filing date	"Y"	document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"L"	document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)	"&"	document member of the same patent family
"O"	document referring to an oral disclosure, use, exhibition or other means		
"P"	document published prior to the international filing date but later than the priority date claimed		

Date of the actual completion of the international search 06 MAY 1998	Date of mailing of the international search report 23 JUN 1998
Name and mailing address of the ISA/US Commissioner of Patents and Trademarks Box PCT Washington, D.C. 20231 Facsimile No. (703) 305-3230	Authorized officer ROBERT SCHWARTZMAN Telephone No. (703) 308-0196

Form PCT/ISA/210 (second sheet)(July 1992)*

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US98/03911

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	BELLAMY et al. Cell death in health and disease: the biology and regulation of apoptosis. Sem. Cancer Biol. February 1995, Vol. 6, No. 1, pages 3-16, see entire document.	1-35

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US98/03911

B. FIELDS SEARCHED
Electronic data bases consulted (Name of data base and where practicable terms used):

STN: MEDLINE, BIOSIS, EMBASE, CAPLUS, WPIDS, JAPIO, PATOSEP, PATOSWO
APS
Search Terms: apoptosis, programmed cell death, inflammation, TNF alpha, cytokine, promoter, enhancer